EFFECTS OF A LOW ENVIRONMENTAL TEMPERATURE ON MICE, WITH ESPECIAL REFERENCE TO COLD RESISTANCE IN YOUNG MICE

BY

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SUMMARY

Mice were bred continuously in an environmental temperature of -3°C, with controls at 21°C. The mice were rigorously inbred and were of the following strains: A (from two sources), GFF and C57BL.

At -3°C all strains showed a marked reduction in fertility and the GFF mice could not be maintained indefinitely at the low temperature. There was a reduction in tail length in the A and C57BL strains in the cold and in the A strain the body weight, body length and total body fat were also reduced. In the C57BL mice there was no significant difference between the body weights or body lengths of experimental and control mice, and the total body fat was reduced less than in the A strains.

The adrenal glands of mice from the cold environment were larger than those of the controls. Adrenal and thyroid glands were examined histologically and histochemically and it was found impossible to distinguish, by inspection, between the organs of 16-week-old mice from the two temperatures.

Experiments were also carried out to study the development of thermo-regulation. Young mice previously kept at 21°C were suddenly exposed to cold. In the strain A mice there were no deaths when 12- or 5-week-old mice were transferred to -3°C, although there was a heavy mortality among mice aged 3 weeks. Many/

Many deaths occurred among 5-week-old mice of the other two strains when treated in the same way. When 3-week-old mice of strain A born and reared at -3°C were placed alone in a cage, a larger proportion of them survived the exposure than mice of the same age and strain transferred to the cold from 21°C. There were no signs of distress among 5-week-old mice of strain A bred at -3°C when they were similarly treated.

The 3-week-old mice of strain A transferred from 21°C fell into two groups which could be readily distinguished after 48 hours' exposure: those which were recovering from the cold exposure and those which were not. The relative adrenal weights of mice which were recovering were lower than those of failing mice and the relative thymus weights were correspond-: ingly greater in the "recovering", that is the "survival", group. The thyroid glands of the "survival" group were very active and their adrenal glands showed no depletion of sudanophilic material after 7 days' exposure. The thyroid glands of failing mice were very inactive and the adrenal cortex was severely depleted. The effects of the sudden exposure to cold on 3-week-old mice were not reproduced at 21°C by injections with either ACTH or cortisone. Neither ACTH nor thyroxine reduced mortality when injected into 3-week-old mice before or during exposure.

The methods of assessment of adrenal and thyroid activity used/

used in the experiments are reviewed and the interaction of these two glands and the mechanism of their control are discussed. The functional development of the pituitary-adrenal and pituitary-thyroid systems and the ability of the animal to increase the rate of carbohydrate metabolism are correlated with the development of control of body temperature. It is con-:sidered that, in normal conditions, this maturation process occurs in strain A mice at or soon after 3 weeks of age, while in mice subjected to a cold environment from birth it appears generally to take place earlier. In the GFF and C57BL mice regulation of the body temperature develops later than in the A strain.

A hypothesis is put forward to explain the difference between the juvenile and adult type of reaction to cold exposure. This difference, it is suggested, depends on the development of a tissue "competence" in respect of the demand for thyroid hormone, which in turn depends on the ability of the tissues to mobilise their glycogen reserves.

CONTENTS

I. INTRODUCTION

The effects of a low environmental temperature on mammals have been the subject of many studies. These can be divided into three main types of investigation:

(a) on Arctic species and problems of insulation;

(b) on physiological responses of species not normally living in a cold environment;

(c) on morphological changes resulting from selection in genetically heterogeneous populations.

Observations on Arctic species have yielded much useful information, particularly since they have demonstrated the morphological and physiological adaptations which occur in mammals which live for long periods of time in a very cold environment. Scholander and his colleagues (1950), for instance, have shown that the Arctic white fox does not increase its metabolic rate (and therefore its heat production) unless the temperature is below $-40^{\circ}C$, while the tropical racoon has a critical temperature of $+25^{\circ}C$, below which its heat production begins to increase. They also showed that the fur is of greater significance for insulation than the skin structure and that the fur of Arctic animals has much greater insulating properties than that of tropical animals. Babanuishev (1934) has demonstrated that effective insulation depends upon the combination of long and dense hairs.

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In some respects, however, information obtained from such studies is of limited value since it cannot always be applied to mammals which have a wider geographical distribution. There are vast numbers of mammals inhabiting the temperate zones which have to withstand considerable fluctuations of environmental temperature and which may be exposed to cold for only short periods. In these animals it is short-term physiological adjustments which are important rather than fixed morphological and physiological "adaptations".

Such short-term physiological adjustments are well known and can be briefly summarized as follows. The initial responses to cold are both neural and hormonal. Those controlled directly by the nervous system include pilo-erection, cardiac acceleration, changes in the vasomotor control of the superficial blood vessels (vaso-constriction of capillaries and alteration of venous flow), and shivering. These combined changes result in a diminution of heat loss and an increase in heat production. The endocrine changes involve the adrenal cortex and medulla, the thyroid, the thymus and the pituitary gland. The adrenal and thyroid glands both undergo a predictable series of changes. The numerous descriptions of those occurring in the adrenal cortex, including that of Selye (1937, 1946) will be dealt with in detail in Sections IV and V. In brief, such changes consist of a short period of marked increase in activity - the "alarm reaction" - followed by a slow/

slow "recovery" stage during which the gland may increase in size. In the adrenal medulla there is an increased production of adrenalin after exposure to cold. The thyroid glands can also be said to show a type of alarm reaction in so far as they have a period of hyperactivity followed by a gradual return to a normal histological appearance. The thymus gland, especially in young mammals, decreases in size after the animal has been exposed to cold. The pituitary gland shows a sudden increase in production of adrenocorticotrophic (ACTH) and thyrotrophic (TSH) hormones immediately after exposure, usually followed by a gradual return to a normal rate of secretion.

Under given conditions the loss of heat by homeothermic animals may be assumed to be proportional to their surface areas. In animals of similar shape and proportions the larger the body the smaller is the surface area relative to it, since volume increases as the cube of the linear dimensions and surface area only as their square. Hence in a cold environment a large body, with a relatively smaller surface area and therefore a reduced heat loss, should be of advantage. According to Bergmann (1847) one might therefore expect to find that the mammals inhabiting the colder regions of the earth were larger than those in the tropics. Similarly, alterations in bodily proportions might be expected in mammals living in the cold, and according to Allen (1877) such differences do occur/

occur between varieties or related species living in different climates. Bergmann's and Allen's "rules", however, have been criticized by Scholander (1955) who has pointed out that some of the workers who have supported them have based their conclusions on data not wholly relevant as a means of estimating the thermal adjustment of the animal. Mayr (1956), however, considers that Scholander's criticism cannot be upheld and that the so-called "climatic rules" of Bergmann and Allen have some justification. In the latter case one might expect that if a population of animals, less specialized than Arctic species, were living in a cold environment there would be a tendency for the average body size to increase through natural selection. This may, in fact, have occurred in the mice studied by Laurie (1946). She published observations on wild house mice (Mus musculus L.) breeding in cold stores kept at about -10°C, and found that these mice were larger than wild mice living at ordinary temperatures. It is unlikely that the original colonizers of these stores were larger or contained more body fat than normal, although this is a possibility. Rather, it may be assumed that they had to make a successful physiological adaptation to this very low temperature before there could be any question of genetical selection.

While many studies on the physiological responses to cold have been concerned with the exposure of animals to low temperatures/

temperatures for relatively short periods of time, very little work has been done on the responses of inbred strains born and reared at a low temperature. A review of the literature also shows that the development of temperature regulation in young mammals presents many problems which, up to now, have been investigated far less than those of thermo-regulation in adults.

The work to be described here is concerned with some of the changes which occur in inbred strains of laboratory mice born and reared at an environmental temperature of -3°C. The changes investigated are related to growth, survival and reproductive performance and with alterations in the adrenal, thyroid and thymus glands.

Two main investigations are described. The first deals, for the most part, with growth, survival and reproduction and with changes in the above-mentioned endocrine glands of mice bred continuously at -3°C, compared with controls at 21°C. The second investigation is concerned with the response of young mice to a low environmental temperature and deals with the effects of sudden exposure to cold on mice aged 3, 5, or 12 weeks.

II. METHODS

(a) Experimental animals

Four strains of mice were used, C57BL, GFF and two strains of A mice. The C57BL and one strain of A mice were obtained from the Imperial Cancer Research Fund (Mill Hill, London), the GFF and the other A strain from Glaxo Laboratories (Greenford, Middlesex). For convenience the A mice will hereafter be termed the A(V) and A(Y) strains respectively.

(b) Experimental conditions

The mice were kept in two sound-proofed constant temperature rooms of identical construction. The cold room was maintained at a temperature of $-3^{\circ}C$, with a differential of $2^{\circ}C$; the temperature occasionally rose above $0^{\circ}C$ when work was going on in the room or when it was being defrosted. The control mice were kept in the warm room at $21^{\circ}C$, with a differential of $5^{\circ}C$. The relative humidity in both rooms was between 60 and 90 per cent. The amount of illumination was the same in each room, artificial light being provided for 12 hours each day; for the other 12 hours the mice were in darkness.

The cages were rectangular metal boxes, 36 x 15 x 11 cm., with wire lids carrying a water bottle and food basket. The floor was covered with sawdust, and cotton wool was provided for/

for nesting. Water could not be kept in bottles in the cold room and was supplied in open jars placed on the floor of the cage. When the water froze the mice licked the ice. The cages were cleaned twice a week unless they contained a litter less than 10 days old, in which case the mice were left undisturbed.

(c) Experimental procedure with breeding stocks

(i) Continuous breeding stocks. Breeding families of the C57BL, A(V) and A(Y) strains were initiated in each case from four litter-mates: one pair in each room. The animals destined for the cold room were first transferred from an animal room (which was kept at about 20°C) to a third constant temperature room maintained at 10°C, for a period of acclimati-: zation of 7 days. These mice were then mated and placed in the cold room, and at the same time their litter-mates were mated and transferred to the warm room. Litters were recorded and counted on the day of birth and were weaned at 21 days. At this time the body weights and sexes were noted and the number of young that had died in the nest was determined.

The mice of subsequent generations that were used for breeding were mated at 5 weeks; all pairs were litter-mates and were left together permanently. Where possible, two, or occasionally three, pairs were mated from each litter. At the second generation it was originally intended to mate only the/

the offspring of the first litters of the initial pairs. Similarly, in the third generation only the first litters of the second generation were to have been mated, and so on to the fourth and fifth generations. In the warm room it was found possible to adhere to this plan, but in the cold room, owing to lowered breeding rates, additional mating was found to be essential in order to maintain the families. Breeding pairs were killed at the age of 30 weeks.

The mice were weighed once a week unless a litter was unweaned, in which case the parents were left undisturbed. The weights of young mice were first recorded, as already stated, at the time of weaning. Many mice were killed at this age in order to save space in the constant temperature rooms. They were killed with ether or chloroform and measurements of head-body and tail lengths were then taken.

Since, as stated above, many of the mice kept in the constant temperature rooms were used for breeding, the number of unmated mice available for experiment was small. These unmated mice were killed at the age of 16 weeks by opening the external jugular veins under ether anaesthesia. Measure-:ments of head-body and tail lengths were taken from the dead animals and the pituitary, thymus, thyroid and adrenal glands were removed and fixed for subsequent weighing and histological investigation. The fixatives used are given in Appendix/

Appendix C. After the removal of these organs, the fat was carefully dissected away from the mesenteries surrounding the abdominal viscera and its fresh weight was determined.

Determinations of total body fat content were made on some of the unmated mice. The animals were killed, the skin removed and cut up into small pieces and homogenised with the carcase. The homogenate was freeze-dried under vacuum and the fat content determined by Soxhlet extraction with chloroform.

(ii) Breeding pairs transferred from -3°C to 21°C. A series of pairs of the A(Y) strain mice that had been reared in the cold room were transferred to the warm room at the age of 5 weeks. These pairs of litter-mates were then mated and their reproductive performance was recorded until the age of 30 weeks. Their breeding record could then be compared with that of mice of the same strain reared and kept at 21°C. For convenience these mice have been called the "reversed" pairs.

(d) Sudden exposure of young mice to -3°C.

Similar numbers of mice of strains A(V) and A(Y) which had been reared at 21°C were taken at the age of 3, 5 or 12 weeks and placed in individual cages at -3°C. They were all provided with nesting material but were allowed no period for acclimatization. Their body weights were recorded before exposure; after periods of 6 hours, 24 hours, 48 hours, 7 days or/

or 21 days they were again weighed and then killed by breaking the neck. Those exposed to cold for more than 24 hours were weighed daily up to 7 days and those exposed for 21 days were then weighed weekly. Organs were removed and fixed as described above in section (c) but the abdominal fat was not removed from these mice.

Litter-mates of some of these mice were killed at the beginning of the experiment, that is at 0 hours, and others were kept at 21°C and killed after 7 days or 21 days. These were the control mice.

A similar series of experiments was carried out with GFF mice aged 5 weeks.

Some 5-week-old C57BL mice were also transferred from 21°C in the same manner to test for survival. That is, the number of deaths was noted and the survivors were killed after 21 days' exposure, but the mice were not weighed and no organs were removed.

Mice of strains A(V) and A(Y) of 3 and 5 weeks born at -3°C were also placed in individual cages for 24 hours, 48 hours, 7 days or 21 days and treated in the same way as described for animals from 21°C. These mice were the -3°C controls.

Experiments involving 3-week-old mice were always begun 24 to 48 hours after weaning, that is, at 22 or 23 days.

III. OBSERVATIONS ON BREEDING STOCKS

(a) Growth

(i) Total growth. Barnett and Manly (1956) found that in three strains, namely A(V), C57BL and GFF, the mice which were transferred from 21°C to -3°C at the age of 8 weeks grew less than their litter-mate controls kept at 21°C. The results with the continuous breeding stocks, that is with mice born and reared in the cold, were, however, slightly different.*

Growth in the A(V) and A(Y) strain mice born and reared at -3°C was reduced in a similar way to that of mice transferred to the cold as adults. That is, from the age of 3 weeks until they were killed at 16 weeks they were lighter than their counterparts bred at 21°C. The C57BL mice, by contrast, showed no significant difference in weight at the two tempera-: tures at 3 or 16 weeks of age, although at 5 weeks the mice reared at -3°C were significantly lighter than the controls at 21°C (Appendix A, Table 1; Appendix B, Fig. 1). The GFF mice were not bred in the cold after the first generation and so there are no corresponding data for this strain.

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*All the figures presented here which concern total body weight in the continuous breeding stocks have been calculated from data from male mice only. At the two temperatures the females showed growth differences similar to those shown by the males but were lighter at all ages.

The difference between the C57BL mice and the two A strains is shown very clearly in a study of the percentage increase in weight. The figures for mice reared at -3°C and 21°C are given below in Table 1.

Table 1

Percentage increase in body weight during various periods in first generation male mice

Strain	Temp.°C	3-5 weeks	5-10 weeks	10-16 weeks
A(V)	21	78.2	48.2	19.0
"	-3	88.3	41.6	11.1
A(Y)	21	94.3	37.1	12.4
"	-3	96.2	46.2	7.6
C57BL	21	114.3	41.1	15.1
"	-3	77.7	62.9	14.8

As is to be expected, all the mice showed the greatest increase in body weight between the ages of 3 and 5 weeks. After this time the rate of growth diminished.

In both the A strains, although the total body weight was reduced in the cold, there was little if any reduction in the rate of growth at the low temperature until after 10 weeks of age/

age. Indeed, between 3 and 5 weeks growth was slightly faster in the cold. In the C57BL strain, on the other hand, the percentage increase in weight between 3 and 5 weeks was considerably lower at -3°C but between 5 and 10 weeks it was higher (62.9 per cent as opposed to only 41.1 per cent at 21°C). Between the ages of 10 and 16 weeks the percentage increase in the C57BL strain was the same in both temperatures.

Estimations of total body fat have so far been made only on 16-week-old female mice of the C57BL and A(Y) strains. The figures for individual mice reared at 21°C or -3°C are given below in Table 2.

Table 2

21°C		-3°C	
Strain	g. fat/100 g. dry homogenate	Strain	g. fat/100 g. dry homogenate
A(Y)	42.93	A(Y)	22.41
"	41.10	"	19.67
"	18.28	"	16.35
"	32.63	"	23.21
"	33.93	"	19.74
"	39.58		
C57BL	30.00	C57BL	21.67
"	26.72	"	24.02
"	30.23	"	23.88

A marked difference was again apparent between the C57BL and the A strains. At 21°C the A(Y) strain females contained more body fat than the C57BL strain and at -3°C the body fat was reduced by about 41 per cent in the A(Y) mice but by only 20 per cent in the C57BL mice.

There was a marked reduction of abdominal fat in both males and females in the two A strains, but no reduction at all in the male C57BL mice (Appendix A, Table 6). There is thus a close relationship between the amount of total body fat and of abdominal fat in the female mice, at least in the two A strains. If it is assumed that determinations of total body fat and of abdominal fat in the males would show similar agreement, some of the observations on body weight could be further analysed. In the A(V) and A(Y) strains much of the increase in weight between 10 and 16 weeks in mice reared at 21°C may be the result of deposition of body fat. If this is the case, the reduced growth at this age in mice reared at -3°C may be partly due to a smaller amount of body fat. In the C57BL strain, on the other hand, the 16-week-old mice bred at -3°C have a lower total fat content than the controls but their body weight is the same. Therefore, it appears that these mice suffer a retardation of growth between the ages of 3 and 5 weeks and then grow at a faster rate than the controls until they are 10 weeks old. At this age the C57BL mice are the same weight as the controls and thereafter grow at the same rate.

(ii)/

(ii) Body and tail lengths. Whatever the change in the amount of body fat may be, it could not entirely account for the difference in weights of the A strain mice at the two temperatures. The length of the body measured from nose-tip to anus showed a marked reduction in the A mice reared at -3°C at both 3 and 16 weeks (Appendix A, Table 2). Male C57BL mice, however, showed no reduction in body length at 16 weeks in the cold. No figures are available for males of this strain at 3 weeks or for the females at any age. It is therefore not yet possible to make a general statement to the effect that a reduction in body weight was always accompanied by a reduction in body length, although the results obtained so far suggest that this is so.

The two strains of A mice differ in tail length. The V strain is short-tailed, whereas the Y strain is "normal" or long-tailed. In each A strain, however, the tail was shorter, relative to body length, in the cold than in the warm (Appendix A, Table 2; Appendix B, Fig. 2). In the C57BL mice the tail length was also markedly reduced at 16 weeks. These results show that alteration in tail length as a result of exposure to cold is independent of change in body length.

(b) Reproduction

(i) Breeding and rearing of young. Reproductive performance at the two temperatures may be compared by reference to/

to the number of young born and weaned per pair, the number of litters born and weaned per pair and the number of young born and weaned per litter (Appendix A, Table 3).

The cold environment caused no increase in the number of barren pairs in any of the three strains, but the number of pairs weaning no young was increased, especially in the A(Y) strain. A few litters were destroyed completely or partly by the parents before they could be counted. Allowance was made for this in calculating the number of young born per litter by omitting these litters from the calculations. No allowance, however, could be made in calculating the number of young born per pair. However, the errors produced are small and the occasions when they occur are indicated in Appendix A, Table 3.

The most important criterion of fertility is the number of young weaned per pair. In this respect there was little difference through four generations between the A(V), A(Y) and C57BL strains at 21°C. At this temperature the mean number of young weaned per pair was 17.7, 20.7 and 20.4 respectively. In all strains fertility was lower at -3°C, where the corresponding figures for the three strains were 6.1, 4.3 and 4.2. The fertility, measured in terms of mean number of young weaned per pair, of the A(V) strain was least affected by the low temperature, showing a reduction of 65 per cent, while that of both the A(Y) and the C57BL strains was reduced by 79 per cent.

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The reduction in the number of young born and weaned per litter was remarkably slight. The main decline in fertility was the result of the large number of whole litters lost. The number of litters born per pair was high in all three strains at 21°C and although this figure was reduced in the cold, the proportion of litters that survived until weaning was very much lower at -3°C than at 21°C.

The results obtained from mice transferred to -3°C at the age of 8 weeks (Barnett and Manly, 1956) agree, for the most part with those described above. In these experiments, however, the fertility of both the GFF and the C57BL strains was reduced by about 90 per cent in the cold, whereas that of the A(V) strain was reduced by only 35 per cent. The fertility of the GFF strain at 21°C, with a mean number of young weaned per pair of 9.8, was lower than that of the other strains the corresponding figures for the C57BL and A strains were 16.0 and 18.5 respectively. Consequently, the fertility of the GFF mice was extremely low at -3°C; the mean number of young weaned per pair at -3°C was 0.9, 1.4 and 12.75 for the GFF, C57BL and A(V) strains respectively.

The transfer of mice to -3°C as adults thus appears in general to result in a greater reduction in both fertility and growth than is observed in mice born and reared in the cold. The low fertility of the GFF strain at 21°C was probably partly the reason why these mice could not be bred indefinitely at -3°C/

-3°C, since a relatively small reduction would be liable to lower the fertility to a level at which the stocks could not be maintained.

(ii) Oestrus. The length and frequency of the oestrous cycle was determined by means of vaginal smears in females born and reared at -3°C and in females transferred to the cold from 21°C as adults. All the mice were observed to have passed through at least one oestrous cycle before the start of the test and litter-mates of mice transferred to the cold were kept at 21°C and used as controls.

In the A and C57BL strains, mice transferred to -3°C as adults showed a marked reduction in the number of oestrous cycles over a period of 4 weeks compared with controls at 21°C (Appendix A, Table 4). It was found that unless the female was just about to be, or was already, in oestrus before transfer to the cold, it remained in dioestrus for 6 to 10 days before the onset of oestrus. After this time the cycles were of a normal length and frequency, namely 3 to 5 days of dioestrus in the A and C57BL mice, as shown by the controls. The reduction in the number of cycles observed over the 4-week period in animals transferred to -3°C was therefore the result of this temporary long dioestrus immediately after exposure. Dioestrus in the GF mice at 21°C, on the other hand, lasted for 8 to 10 days and no reduction was observed after transfer to the cold.

Observations/

Observations on A strain mice born and reared at -3°C, compared with those on controls at 21°C, indicate that there was no significant difference between the number of oestrous cycles over a period of 14 days in the two groups of animals (Appendix A, Table 5).

It seems that in mice reared at -3°C the oestrous cycle is unaffected by the cold environment, and that the same applies to mice transferred to the cold as adults but only after 11 days' exposure. The long dioestrus in the GFF mice at 21°C may account for the low fertility observed in the control mice of this strain. Disturbances in the oestrous cycle, however, do not appear to be the primary cause of the lowered fertility observed in all the mice at -3°C.

(c) Long-term effects on reproduction and growth

There was never any indication that the second and subsequent generations of young born in the cold were better adapted than the first generation (born of parents reared in the warm). The total growth of second and third generation mice was similar to that of the first (Appendix A, Table 1) and the same was true of the body and tail lengths.

The reproductive performance of the "reversed" pairs, that is the A(Y) strain mice transferred from -3°C to 21°C at the age of 5 weeks, was very similar to that of mice of the same strain in the warm. The results obtained from these reversed pairs are given below in Table 3 together with the figures for mice born and reared at 21°C.

Table 3

	Reversed	21°C
No. of pairs	7	15
Mean no. young born/pair	24.43 ± 2.32	27.13 ± 2.96
Mean no. young weaned/pair	18.00 ± 2.64	20.67 ± 2.50
Mean no. young born/litter	5.18 ± 0.36	5.29 ± 0.20
Mean no. young weaned/litter	3.82 ± 0.44	4.70 ± 0.22
Mean no. litters born/pair	4.71 ± 0.34	5.13 ± 0.54
Mean no. litters weaned/pair	3.57 ± 0.34	4.40 ± 0.52

Although all the figures for the reversed pairs were slightly lower than those for the pairs born at 21°C, the fertility, measured in terms of the mean number of young weaned per pair, was not significantly reduced in the reversed mice. These results, therefore, do not give any indication of a long-term genetical alteration in the breeding stocks at -3°C, since after transfer to 21°C the reproductive performance of these mice was comparable to that of mice born and reared in the warm.

(d)/

(d) Changes in endocrine glands

(i) Weights. The weights of the adrenal and thymus glands of the unmated mice are given in Appendix A, Table 6.

There was an increase in the relative adrenal weights of mice reared at -3°C compared with controls at 21°C, and this was especially obvious in the A(V) strain. This increase was statistically significant in all groups except the A(Y) females. Although growth and reproduction at both temperatures was very similar in the A(V) and A(Y) strains, they showed a marked difference in the amount of adrenal hypertrophy at -3°C. In the A(V) males, the mean relative adrenal weight at 21°C was 4.53 mg./100 g. while at -3°C it was 10.02 mg./100 g., an increase of 121.2 per cent. In the A(Y) males, on the other hand, the corresponding figures were 4.22 mg. and 6.56 mg., an increase of only 55.5 per cent. In the females this difference between the strains was even more marked. The mean adrenal weight of the A(V) females was increased by 118.3 per cent in the cold, while that of the A(Y) females was increased by only 13.6 per cent.

Except for the A(V) females, there was no significant difference between the thymus weights of mice aged 16 weeks at the two temperatures, although those of mice at -3°C tended to be lower. In the A(V) females, the relative thymus weights were significantly heavier at -3°C than at 21°C, but no explanation can be given for this surprising result.

It was found impossible to make accurate determinations of thyroid weight in these experiments. The gland appeared to be entirely free of muscle when it was removed under a lens. However, on examining sections under the microscope, a very thin but variable sheet of muscle was frequently found to be adhering to its lateral lobes underneath the outer sheath of connective tissue. Attempts to remove this sheath and the underlying muscle invariably resulted in damage to the gland. Examination of the thyroid glands was therefore confined to their histological appearance.

(ii) Histological effects. Histologically, the adrenal and thyroid glands of A strain mice from the two temperatures were indistinguishable. There was no evidence of any depletion of sudanophilic substance from the adrenal cortex of mice kept at -3°C either in the unmated mice (Appendix B, Figs 3, 4) or in the breeding pairs (Appendix B, Figs 5, 6). The thyroid glands of mice reared at -3°C showed on inspection a normal incidence of moderately active and inactive follicles (Appendix B, Figs 7, 8).

(e) Discussion

These results show that although the A and C57BL strains will probably breed indefinitely in the cold, the reproductive performance and growth of all the strains were markedly affected by the low temperature.

(i)/

(i) Thermal relationships. The advantages of a large body size to a mammal living in a cold environment have already been discussed in the Introduction. In this section it was also mentioned that Laurie (1946) found that wild mice living in cold stores were larger than wild mice living at ordinary temperatures. This may have represented a genetical difference and in the cold conditions the increase in size may have been an advantage. In the experiments described above, however, with the exception of the C57BL strain at 3 and 16 weeks, mice bred at -3°C were lighter at all ages than control animals kept at 21°C. Even the C57BL mice reared at -3°C did not grow larger than the controls. These results are in agreement with those of Retzlaff (1939) who reported that adult white mice living in a cold environment were lighter than those living at normal temperatures.

Thus inbred laboratory mice show no ability to adapt themselves to a cold environment by increased total growth.

In the present experiments it was found that after a retardation of growth, the C57BL mice in the cold grew at a faster rate than the controls for several weeks before reverting to a normal growth rate. This type of over-compensation after a set-back is quite common. Clarke and Smith (1938) subjected young albino rats to varying periods of semi-starvation by restricting their intake of energy-producing food or of mineral salts/

salts. This treatment produced a marked suppression of growth. When the animals were later given a full diet, Clarke and Smith found that those given the restricted diet for the shortest period, namely 3 weeks, over-compensated by exceeding the growth of the control rats. Rats stunted by either method for longer periods failed to regain losses suffered during suppressed growth. A similar effect was reported by Barnes et al. (1947) who studied the growth of normal rats and of rats stunted by a restricted diet for 330 or 900 days. They found that when the animals were given a full diet the initial rate of increase of the ratio of body weight to tibia length (g. per cm.) was much greater than the rate of increase of this ratio for the control rats at any time during their lives.

Another type of adaptive growth change which might be expected to occur in mice living in a cold environment is an alteration in bodily proportions. It has been mentioned in the Introduction that according to Allen (1877) such differences do occur between related species living in different climates. Sumner (1915) reported that rearing mice in the cold led to a reduction in the weights and lengths of feet, ears and tails. In the present experiments no evidence has been obtained so far of any reduction in the lengths of feet or ears among mice reared at -3°C, but, as stated above, the tail length was reduced Whether this has any adaptive significance in terms of heat conservation is not established. Barnett (1956) showed that the/

the surface body temperature of mice living at -3°C was about 30°C. The amount of heat loss via the tail, therefore, must be small compared with the heat loss over the whole surface of the body. A reduction in tail length of the amount observed in these experiments is unlikely to involve any substantial reduction in the area over which heat loss occurs. The means by which the reduction is brought about is a separate question: the shorter tails of mice reared in the cold may be a result of prolonged vaso-constriction in the tail during early infancy.

In general it seems that the main effect of the growth changes observed is likely to be disadvantageous: a reduction in size at a low temperature means that the animal will produce still more heat than would be necessary if its size were unchanged.

(ii) Reproduction. There is little published information on the effects of a low environmental temperature on reproduction in mammals. Lee (1926) found that the oestrous cycle in albino rats was lengthened by exposure to outdoor winter temperatures but, according to Parkes and Brambell (1928), the transfer of laboratory mice from an environment of about 18°C to one of about 0°C led to only a temporary disturbance of the oestrous cycle. These results of Parkes and Brambell have been confirmed by observations on all strains of mice used in the/

the experiments described in Section 3(b) and, in addition, it has been found that the oestrous cycle of A strain mice reared in the cold is normal.

It is possible that the fertility of the males is affected, since although the testes of the mice in the cold do not descend to the same extent as those of the control animals, the scrotal temperature may be below the level for maximum fertility. Bogart and Mayer (1946) have shown, however, that the effects of heat on reproduction in rats are due to changes in thyroid function and not to direct effects on the testes. It is therefore possible that the low temperature has no direct effect on the testes either.

The results obtained from the "reversed" breeding pairs, that is those transferred from -3°C to 21°C at the age of 5 weeks, indicate, as expected, that after several generations in the cold the experimental mice are not substantially different in reproductive ability from those in the warm. There is no reason to think that any major genetical change occurred in the mice at -3°C. The reason for the fewer number of litters born in the cold is difficult to determine, especially since the oestrous cycle was unaffected. A large number of the deaths during infancy observed among the mice living at -3°C must be due to the direct effect of the cold on the young mice themselves.

(iii)/

(iii) Endocrinology. The fact that the thyroid glands are essential for the survival of mammals exposed to cold has been known for many years, and recently Barrow and Money (1949) and Ershoff (1948) found that thiouracil-treated rats lose their ability to survive in a cold environment. Leblond and Gross (1943) showed that thyroidectomy greatly shortened the survival period of adult rats exposed to temperatures of 0°C to -2°C, although the immediate reactions to cold of increase in metabolic rate, of food consumption and of adrenal enlargement were comparable to those of normal animals, and so were not mediated entirely by the thyroid. These results agree with the findings of Sellers and You (1950) who attributed part of the immediate rise in the metabolic rate to muscular exercise. Insufficient evidence has been obtained so far from the mice reared at -3°C to determine whether they show any sustained increase in muscular activity. The work of Hart (1952) on mice and Hart and Heroux (1955) on lemmings and rabbits, indicates that it is unlikely that activity is increased in the cold. These authors found that exercise in cold environments can have a deleterious effect on temperature regulation, since work involving muscular exercise tends to decrease insulation. As a result, much of the heat produced by the work may not be available for maintaining body temperature in a cold environment.

The increased activity of the thyroid glands in mammals exposed/

exposed to cold has been described in great detail in many papers, including those of Baillif (1937) and Catz et al. (1953). Turner (1946) showed that the thyroid glands of mice increase in size after exposure to cold. This increase in size was used by Dempsey and Astwood (1943) to measure the increase in thyroid hormone production which occurs with a lowering of the environmental temperature. They measured the amount of exogenous thyroid hormone that was necessary to prevent hyper-: trophy of the thyroid glands of thiouracil-treated rats. Stevens et al. (1955) found that the rate of release of thyroidal radio-iodine in guinea-pigs exposed to temperatures of 5°C to 9°C was approximately doubled.

Leblond et al. (1944), using rats injected with radio-iodine, showed that after 40 days' exposure to temperatures of 0°C to 2°C, the iodine metabolism was back to control levels and the histological appearance of the thyroids was similar to that of the controls. Starr and Roskelly (1940) also showed that the epithelial changes and loss of colloid in the thyroids of rats exposed to 12°C returned to normal and the gland showed an involution of hypertrophy after about 45 days' exposure. These results are in conformity with the normal appearance of the thyroid glands of the mice living in the cold in the experiments described above.

Prolonged exposure to cold also causes an increase in the weight/

weight of the adrenal glands, as observed in the present experiments. Although most authors find that the increase takes place in the cortex, Morin (1946) and Schaeffer (1946) consider that the medulla also is enlarged. This point has not yet been examined in the material collected in the present experiments.

(iv) Cold resistance in young and infant mammals. It has been stated above that the cold environment may have been the direct cause of much of the mortality among infant mice living at -3°C. The effects of a low temperature on mice of this age, however, cannot be judged on the basis of the physiological changes which occur in adults. The changes which take place in the thyroids and adrenals and the raised metabolic rate which typically occur when an adult mammal is subjected to cold are not always observed in infant mammals. Several workers have shown that rats and mice are poikilothermous at birth.

Fitzgerald (1955) found that the oxygen consumption of newborn mice increases with a rise in the environmental tempe-: rature from 0°C to 5°C. He showed that, at these temperatures, all the oxygen needed by the animal could be obtained by simple diffusion, and that no gross respiratory movements were observed until the temperature rose to 11°C. Fairfield (1948) cooled 0- to 17-day-old rats to temperatures ranging from 20°C to 2°C and observed an immediate fall in intraperitoneal temperature/

temperature. He also showed that rats up to 10 days of age could be cooled until their metabolic rate (measured by oxygen consumption) fell to zero and remained so for 108 minutes, and that when re-warmed they showed a complete recovery. The work of Adolph (1948, 1951) indicates that these observations were the result of a combined tolerance of young rats to both cold and anoxia. Adolph also demonstrated that tolerance to anoxia is lost before that to cold. Hill (1947) found that the ability of white rats to control their body temperature was not developed until the 18th day after birth. He found that during the period from the 18th to the 30th day there was rapid development of resistance to cold and that this was followed by a slow improvement in temperature control up to the age of 60 days. Capek et al. (1956) and Hahn (1956) have shown that in rats the development of effective thermo-regulation depends on the ability of the liver and muscles to release stored glycogen on exposure of the animal to cold, and that this develops only at 18 days.

It is probable that, like rats, infant mice can tolerate a fall in body temperature. Barnett (1956) showed that mice born at -3°C are exposed to low nest temperatures at frequent intervals during infancy. They begin to regulate their body temperature before the age of 21 days, but not at first in so efficient a manner as adults.

These/

These results indicate that one of the most critical times in the life of the mice exposed to cold in the present experiments must be expected to be at the age of 21 days, when they are separated from their parents. By this time they have presumably lost their tolerance to cold and when they are separated from their parents they are deprived of an important source of heat and might be expected to suffer severely from cold exposure. A number of deaths among the mice bred at -3°C did occur at this time. There were, however, no deaths in strain A when, at 5 weeks, the unmated members of a litter were separated, even if one individual was kept alone in a cage.

It is evident that individual size and numbers in a litter are not the only factors determining survival in the cold and that some physiological adaptation occurs between the ages of 3 and 5 weeks, similar to that described for rats.

IV. OBSERVATIONS ON YOUNG MICE

As stated above in Section 2(d), mice of both the A(V) and the A(Y) strains were used in the experiments designed to study some of the effects of sudden exposure to cold in young mice. The experiments already described have indicated slight growth differences between these two strains at the age of 16 weeks at both 21°C and -3°C and a marked difference between relative adrenal weights in the two strains reared in the cold. No difference in relative adrenal weight, however, was evident in mice aged 3 or 5 weeks. In the experiments involving transfer of mice aged 3, 5 or 12 weeks from 21°C to -3°C, the changes observed in the body weights and relative organ weights were almost identical in the A(V) and A(Y) strains. For convenience, therefore, the figures given in the Tables in Appendix A are calculated from the combined results of the two strains. Selected examples of the figures obtained from the individual strains are given in Appendix A, Table 14. Owing to the small numbers of mice, it is not yet possible to state that at no point were the differences statistically significant. However, any differences observed in these young mice between the two strains at a given temperature were very much smaller than the differences found between temperatures.

Mice of all three age groups that were transferred to the cold from 21°C suffered an initial fall in body weight. This

was/

was probably the result of a loss of body fat, since the amount of abdominal fat was seen on inspection to be greatly reduced.

(a) 12-week-old mice

There were no deaths among the mice aged 12 weeks. These mice had almost stopped growing before the experiment began and the males did not regain the lost weight; that is, after 21 days' exposure their weights remained about 3 g. below their mean initial weight of 28.3 g. The females, however, had nearly regained their initial weight (mean value 23.8 g.) after 21 days.

The growth of the control animals, that is those kept at 21°C, was so slight that the organ weights of both experimental and control mice are expressed in terms of mg./100 g. of the initial body weight (Appendix A, Table 7). During the experiment the thymus glands of mice of both sexes decreased in weight relative to body weight and the adrenal glands increased.

In both sexes the thymus glands were significantly reduced after 7 days' exposure by comparison with those of the 12-week-old controls; the mean thymus weight of the males was reduced by 47 per cent and that of the females by 41 per cent. After 21 days the thymus weights were still significantly different from those of the controls aged 15 weeks although, especially in the females, the difference was less than at 7 days/

days. After 21 days' exposure the mean thymus weight of the males was reduced by 41 per cent and that of the females by only 26 per cent.

The adrenal glands of both sexes were for some time not significantly heavier than those of the controls. Between 7 and 21 days, however, the difference became significant and after 21 days' exposure the mean adrenal weight of the males was increased by 36 per cent and that of the females by 59 per cent.

The histology of the adrenal glands also changed as a result of exposure to cold; in particular, the zona fasciculata of the cortex was markedly affected. The changes in the adrenal cortex of a mammal exposed to cold are illustrated diagrammatically in Appendix B, Fig. 9. Fig. 9(a) represents a sector of a normal adrenal gland; the stippled area represents the cortex, with an outer zona glomerulosa and a wide inner zona fasciculata; the innermost zone of the cortex, the zona reticularis, has been omitted and the unshaded region represents the medulla. The degree of stippling indicates the concentration of lipid material which readily takes up Sudan stains. Fig. 9(b) represents the appearance of the gland after about 6 hours' exposure to cold; the immediate reaction is depletion of the sudanophilic substance. Fig. 9(c) represents a gland after about 36 hours' exposure: further

lipid/

lipid depletion occurs with hypertrophy of the zona fasciculata. This is described by Selye (1937, 1946) as the "alarm reaction". Fig. 9(d) represents that of an animal which died during the experiment; the lipid depletion in such cases is often extreme and according to Selye maximum hypertrophy may occur during this phase. The question whether this should be described as an "exhaustion" phase will be discussed later. Fig. 9(e) represents a gland after about 7 days' exposure. During the "resistance" or "recovery" stage the lipid re-accumulates in the fasciculata and may be present in supra-:normal amounts. The zona fasciculata at this stage shows considerable hypertrophy.

The adrenals of all 12-week-old mice which were killed after 6 to 48 hours' exposure showed the typical "alarm" reaction; all those killed after 7 or 21 days were found to be in the "recovery" stage (Appendix B, Figs 10, 11). The maximum hypertrophy, indicated by increase in relative weight of the whole gland, was observed after 21 days' exposure, showing that the extra growth of the gland continued after the re-accumulation of the sudanophilic substance within the fasciculata.

The thyroid glands of these mice became more active immediately after exposure (Appendix B, Figs 12, 13). As the photomicrographs illustrate, the follicular epithelium was heightened and the nuclear volume increased. In addition, the colloid/

colloid became less basic and the change in pH was clearly indicated by use of the Masson trichrome stain. The colloid of a resting follicle stained red, that of an active follicle, green. The thyroid glands of 12- or 15-week-old control mice, kept at 21°C, contained a mixture of active and inactive follicles. The active ones were mainly in the outer part of the gland. After the mice had been exposed to cold nearly every follicle was found to contain colloid which had taken up the green stain.

This increase in activity of the thyroid glands was, as might be expected, still apparent after 21 days' exposure.

(b) 5-week-old mice

(i) Strain A. 5-week-old mice of the A strain which were transferred to the cold from 21°C showed the initial decline in weight but began to grow again after 48 hours' exposure (Appendix A, Table 8; Appendix B, Figs 14, 15). After 21 days they were heavier than at the start of the experiment, although they had much less abdominal fat. There was one exception to this statement, namely a male, which will be referred to again later. There were no deaths in this group of 5-week-old mice.

The control animals grew very little in 48 hours. The organ weights of mice killed after 0, 6, 24 and 48 hours' exposure are therefore expressed in terms of mg./100 g. of the initial/

initial body weight. After 7 days, however, the experimental animals had begun to grow again, although their total body weight remained less than that of the controls at the same age. This difference was partly due to the fact that, as stated above, after transfer from 21°C to -3°C a considerable amount of weight was lost through consumption of body fat. This fat was not replaced during the experimental period. Hence the weight of a small organ, expressed as a proportion of the final body weight after 7 or 21 days' exposure, would give a mis-:leading impression of hypertrophy in the organ. To allow for this it would be desirable to express each organ weight as a proportion of body weight less fat reserve. The weight of the fat reserve in these mice was, however, unknown. Instead, an allowance was made by determining the mean initial decline in weight which occurred among experimental mice of the same age, sex and weight as the control mice when killed at the end of the experiment. The figure of mean loss was expressed as a percentage of the initial body weight of the experimental mice: for males, both at 7 and 21 days, the loss was 6 per cent; for females it was 9 per cent. The body weights of the control mice used in the calculation of relative organ weights were therefore the actual weights less 6 and 9 per cent respectively. It was then possible to make a direct comparison of the relative organ weights, expressed in terms of mg./100 g. of the final body weight, of experimental and control/

control animals after 7 and 21 days' exposure without the complication of the difference in body fat.

The actual body weights of the control mice at 7 and 21 days are given below in Table 4, together with the mean weight loss among the corresponding experimental mice. The percentage weight loss is called the "allowance". The standard deviations are given in brackets.

Table 4

	Males		Females	
	7 day	21 day	7 day	21 day
No. of mice at 21°C	11	5	10	5
Mean final wt. in g. at 21°C	21.5	21.6	17.8	18.9
No. of mice at -3°C	9	9	7	6
Mean wt. loss in g. at -3°C	1.35(0.5)	1.35(0.5)	1.65(0.6)	1.75(0.5)
Allowance (%)	6	6	9	9

In Appendix A, Table 8, the actual figures of the relative organ weights are given as well as the adjusted ones. The occasions/

occasions when the differences between the relative organ weights of the experimental and control mice are not significant with the actual figures, but are significant with the adjusted ones, are indicated in the Table. It seems probable that the adjusted figures represent the true weight changes better than the unadjusted figures.

The adrenal glands increased and the thymus glands decreased in weight in much the same way as described for the 12-week-old mice and similar differences were observed between the two sexes.

In the males the mean relative thymus weight decreased steadily and was significantly different from that of the controls after 7 days' exposure. It was reduced by 16 per cent at 48 hours, 37 per cent at 7 days and by 43 per cent at 21 days. The corresponding figures for the females were 37 per cent, 34 per cent and +1.6 per cent (the last being an increase). The difference between the thymus weights of the experimental and control female mice was highly significant after only 48 hours' exposure but thereafter the thymus glands of the experimental mice apparently began to regenerate and by 21 days were actually heavier than those of the controls.

The adrenal hypertrophy was again more pronounced in the females, in which the mean adrenal weight was increased by 50 per cent after 21 days' exposure. In the males it was increased by only 30 per cent and did not show a significant difference.

This difference between the males and females is clearly illustrated in the graphs shown in Appendix B, Figs 14, 15.

In both sexes the alteration in the histology of the adrenal glands was very similar to that described for the 12-week-old mice, although the pattern of depletion was variable. In every mouse examined after 6, 24 or 48 hours' exposure the adrenal glands showed the typical "alarm reaction" and, with one exception, the mice killed after 7 or 21 days were found to be in the "recovery" stage (Appendix B, Figs 16, 17).

In order to investigate the nature of the sudanophilic material in the adrenal cortex pairs of adjacent sections were taken from 14 adrenals; one of each pair was stained with Sudan black and the other with the Schultz stain for cholesterol In all adrenals the area in which the respective stains were taken up was identical. This was especially noticeable with adrenals in which the cortex was partially depleted of lipid, when the pattern of depletion was the same in the two sections (Appendix B, Figs 18, 19). These results indicate that the sudanophilic substance or lipid of the zona glomerulosa and zona fasciculata is almost certainly cholesterol. The lipid content of the degenerating X zone of the females did not react to the Schultz stain, showing that this zone does not contain cholesterol at this stage.

The thyroids became active immediately after exposure and remained active throughout the 21-day experimental period (Appendix B, Figs 20, 21).

The one mouse (a male) which failed to regain its initial weight continued to lose weight steadily; when it was killed after 7 days it had fallen from 17.5 g. to 13.5 g.. Its thymus gland was reduced to 31.1 mg./100 g., a figure far below the mean (89.1 mg./100 g.), and its adrenal was enlarged above the mean of 6.04 mg./100 g. to 10.4 mg./100 g.. The adrenal cortex of this mouse was heavily depleted and its thyroid gland showed no sign of increased activity (Appendix B, Figs 22, 23). The thyroid was, in fact, less active than that of a typical control animal. The significance of the response of this mouse will be discussed later (Section VI).

(ii) Strain A, -3°C controls. The 5-week-old mice of the A strain born in the cold room all survived after they were placed alone in a cage. Such a result was to be expected since all the 5-week-old A strain mice reared at 21°C survived the transfer to -3°C. Further, the mice born in the cold showed no fall in weight and no depletion of the adrenal cortex. In short, there were no evident signs of distress.

The mice grew rapidly during the experiment. Their mean initial weight, 15.4 g. in the males and 12.5 g. in the females, was less than that of their counterparts from the warm room, with/

with a mean initial weight of 19.0 g. and 16.8 g. respectively. In addition, their bodies contained much less body fat. The results of this experiment are recorded in Appendix A, Table 9.

All the organ weights are expressed in terms of the final body weight. The relative organ weights did not alter significantly during the experiment. The thymus glands of both sexes tended to decrease in weight but the relative adrenal weights only increased in the females and even then the increase was not statistically significant at $P = 0.5$.

The histological appearance of the adrenal glands did not change during the experiment (Appendix B, Figs 24, 25) but the thyroids showed an increased activity in 9 out of 12 mice.

(iii) Strain GFF. Of the 5-week-old GFF mice transferred from 21°C to -3°C, 18 per cent died before the end of 24 hours' and 23 per cent before the end of 21 days' exposure. The GFF mice showed the usual fall in body weight but by 7 days most of the survivors had regained the lost weight and by 21 days they were heavier than at the beginning of the experiment (Appendix A, Table 10).

The organ weights were calculated in the same way as those of the 5-week-old A strain mice, that is, up to 48 hours' exposure as mg./100 g. of the initial body weight and at 7 and 21 days as mg./100 g. of the final body weight. As in the case/

case of the A strain mice, an allowance was made for the greater amount of body fat in the 7- and 21-day control animals by subtracting a percentage from the actual body weights of the controls and using the adjusted figures in the calculation of the relative organ weights (Table 5).

Table 5

	Males		Females	
	7 day	21 day	7 day	21 day
No. of mice at 21°C	5	5	5	5
Mean final wt. in g. at 21°C	22.9	24.6	20.5	22.1
No. of mice at -3°C	8	5	6	5
Mean wt. loss in g. at -3°C	2.04(0.6)	2.22(0.8)	2.09(0.6)	2.00(0.7)
Allowance (%)	9	9	10	9

Standard deviation given in brackets

Both the adjusted and unadjusted figures for the relative organ weights are given in Appendix A, Table 10.

The response of the GFF mice aged 5 weeks to sudden exposure/

exposure to cold differed from that of the A strain mice of the same age in several ways. In the first place, as already mentioned, they suffered quite a high casualty rate. The other differences are reflected in the rate of change of their relative organ weights.

The fall in weight of the thymus glands was more rapid than in the A strain mice. In the males the thymus glands of the A strain were not significantly reduced until the 7th day, at which time the mean thymus weight was 37 per cent less than that of the control mice. In the GFF strain, on the other hand, the thymus glands of the males were significantly reduced by 35 per cent after only 48 hours' exposure. In the females the change in thymus weight was similar in both strains, that is, there was a significant reduction after 48 hours' exposure. However, whereas the thymus glands of the A strain mice began to regenerate after 48 hours, those of the GFF females did not do so until after 7 days. The mean thymus weight of the latter was therefore less than that of the controls even after 21 days, although the difference was not statistically significant.

A difference between the A and GFF strains was also apparent in the change of relative adrenal weight. In both the 12- and 5-week-old A strain mice transferred to -3°C from 21°C, the maximum hypertrophy of the adrenal glands occurred after/

after 21 days' exposure. In the GFF mice the increase in size was more rapid and in the females the maximum hypertrophy occurred after only 7 days' exposure. In addition to this difference in change of weight the adrenal glands of the GFF mice were heavier than those of the A strain aged 5 weeks; the mean adrenal weights at 0 hours expressed as mg./100 g. were, in the GFF mice, 8.6 for the males and 11.5 for the females and in the A mice, 6.9 and 8.9 respectively.

The histological appearance of the adrenal and thyroid glands was very similar to that of the A strain mice. The adrenals exhibited a partial depletion of lipid in the zona fasciculata up to 48 hours' exposure but by 7 days had reached the recovery stage (Appendix B, Figs 26, 27).

(iv) Strain C57BL. C57BL mice were transferred from 21°C to -3°C to test for survival in these conditions; the survivors were killed after 21 days but no organs were removed. 15 males and 14 females were used and of these, 7 males and 7 females, that is 48 per cent, died before 4 days' exposure. There were no further casualties and the survivors appeared to have adapted themselves completely by the end of 21 days.

(c) 3-week-old mice

(i) Strain A. Of the 3-week-old mice born in the warm and transferred to -3°C, 44 per cent died before 7 days' exposure/

exposure. Only 1 mouse, a female, had died by 6 hours; most of the deaths occurred between 24 and 60 hours. Only 1 male and 3 females out of the 6 males and 12 females which had been intended for 21 days' exposure survived for the full period.

After 48 hours' exposure these mice fell into two distinct groups: one group continued to lose weight and seemed to be slowly dying; the other group appeared to be recovering and had regained the initial loss of body weight.

The organ weights were calculated in the same manner as were those of the 5-week-old mice (Appendix A, Table 11; Appendix B, Figs 28, 29). The body and organ weights of the two, apparently different, groups are tabulated separately, (a) being the "failure" group and (b) the "survival" group. An allowance was made for the greater amount of body fat in the 7- and 21-day control animals in the same way as described for the 5-week-old mice (Table 6).

Table 6/

Table 6

	Males		Females	
	7 day	21 day	7 day	21 day
No. of mice at 21°C	5	11	6	10
Mean final wt. in g. at 21°C	15.9	21.5	13.5	17.8
No. of mice at -3°C	12	9	11	7
Mean wt. loss in g. at -3°C	1.75(0.6)	1.35(0.5)	1.60(0.5)	1.65(0.7)
Allowance (%)	11	6	12	9.5

Standard deviations given in brackets

After 7 days' exposure the difference between the two groups was very obvious.

By the 7th day the body weights of the "failure" group females were significantly lower than at the beginning of the experiment but those of the males were very variable and no significant difference emerged. All the mice showed a significant fall in thymus weight after 48 hours' exposure, and by 7 days the difference between the two groups was so marked that the absolute values of the relative thymus weights did/

did not overlap. Taking the females as an example, at 7 days both groups showed a significant fall in thymus weight by comparison with the controls. The mean thymus weight of the control mice was 296.5 mg./100 g. and that of the "survival" group (b) 199.9 mg./100 g., a reduction of 33 per cent. The mean thymus weight of the "failure" group (a), on the other hand, was reduced by nearly 87 per cent to 39.8 mg./100 g..

In both sexes the adrenal weights of the "survival" group were comparable with those of the controls, while the adrenal glands of the "failure" group were significantly heavier.

The histology of the adrenals and thyroids was markedly different in the two groups. All the adrenals showed depletion of the zona fasciculata at first but at 7 days the difference between the two groups was obvious. The group (b) mice were found to be in the typical "recovery" stage, that is the lipid had returned to the adrenal cortex. The thyroid glands of this group were still very active, showing columnar follicular cells with large round nuclei and a colloid that stained green with Masson's trichrome stain (Appendix B, Figs 30-35). The adrenals of the "failure" group (a) mice were found, at 7 days, to be heavily depleted. The thyroid glands of these animals were very remarkable in that they appeared to be inactive. The follicular cells were flattened, the nuclei were ovoid and the colloid was hard and took up the red rather than the green stain with Masson's trichrome stain (Appendix B, Figs 36-38).

(ii)/

(ii) Changes in organs of mice which died during exposure.

The mice of the "failure" group have been described above as 'appearing to be slowly dying', not merely because they looked ill and were losing weight but because the histology of their thyroids and adrenals showed a close resemblance to that of animals which had, in fact, died. This statement is based on the study of the organs from three 3-week-old mice which died during the experiment; the organs of the other mice that died were not examined owing to the speed at which the bodies were frozen and so made unsuitable for histological study. After being thawed out the thyroids especially were found to be in a poor condition.

The adrenals of the mice that died after 2 to 7 days' exposure were very severely depleted and were in the condition illustrated in Section (d) of Appendix B, Fig. 9. The thyroids were apparently very inactive, the follicular cells being almost entirely of the pavement type and the nuclei very flat-: tened (Appendix B, Figs 39-41).

(iii) Strain A, -3°C controls. The 3-week-old mice born in the cold showed a mortality of only 20 per cent compared with the 44 per cent among those transferred from 21°C. The changes in body weight during the experiment were variable: most of the mice grew quite rapidly but growth was checked in a few. The relative organ weights were all calculated from the final body weights (Appendix A, Table 12).

The/

The organ weights were variable but, as with the 5-week-old mice born at -3°C, no significant difference was found between the relative organ weights of the mice killed at 0 hours and those of the mice treated in the same way as those from 21°C for the specified periods. The relative thymus weights did tend to decrease but the relative size of the adrenal glands was almost unchanged after 7 days' exposure. The initial thymus weights were much lower than those of the mice reared at 21°C. The mean initial thymus weights, expressed as mg./100 g. of the mice bred at -3°C, were 192.8 in the males and 196.2 in the females; the corresponding figures for mice reared at 21°C were 269.0 and 281.9 for males and females respectively. In the males the adrenals were heavier than those of the 3-week-old mice from the warm room.

All the mice killed after 24 or 48 hours' exposure showed depletion of the adrenal cortex but only one mouse, a male, was not in the typical "recovery" stage by 7 days (Appendix B, Figs 46, 47). The thymus of this male was reduced to 36.0 mg./100 g. (compared with the mean figure of 116.6 mg./100 g.) and its adrenal was much enlarged to 17.3 mg./100 g. (the mean figure being 11.2 mg./100 g.). The zona fasciculata was heavily depleted. Its thyroid glands were inactive while those of the rest of the mice were extremely active, especially after 7 days when the animals were growing rapidly.

(d)/

(d) Assessment of thyroid activity by means of autoradiographs.

An additional check was made on the activity of the thyroids of 3-week-old mice by means of radio-active iodine. 10 to 30 microcuries of I^{131} were injected into 3-week-old mice which had been exposed for 7 days, into 5-week-old mice which had been exposed for 48 hours and into control mice of both ages which had been kept at 21°C. The animals were killed 6 hours after the injection and the thyroid, thymus and adrenal glands removed. The thyroids were fixed in 10 per cent formaldehyde, embedded in paraffin and autoradiographs were made from a series of sections from each block.* Adjacent sections were labelled and stained by routine proce-:dures.

The body weights and some of the organ weights of the 3-week-old mice are given with the results from the autoradio-:graphs in Table 7.

Table 7/

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* I am indebted to Dr. S. Kennedy (Pathology Department, Royal Infirmary, Glasgow) for the injection of the mice and the preparation of the autoradiographs.

Table 7

3-week-old mice exposed for 7 days					
Sex	Initial wt. g.	Final wt. g.	Adrenal wt. mg./100 g.	Thymus wt. mg./100 g.	I^{131} concentration
♀	10.5	9.5			high
♀	10.5*	8.5*			low*
♀	13.0	13.0			high
♀	11.5	13.0	12.3	104.6	high
♀	12.0	15.5	12.3	144.5	high
♂	11.0	12.5			high
♂	12.0	13.5	9.6	122.2	high
♂	12.0*	11.0*	12.7*	27.3*	low*
♂	10.5*	10.0*	12.2*	40.0*	low*
♂	12.0	14.5	8.3	127.6	high
3-week-old mice kept at 21°C for 7 days					
♀	10.0	15.0			high
♀	13.0	15.0			high
♂	10.5	15.5			high
♂	11.0	16.5	8.5	133.3	high

The concentration of radio-active iodine in the 3-week-old mice, otherwise classed as members of the "failure" group and indicated by an asterisk in Table 7, was strikingly different from that in mice falling into the "survival" group. For this reason it did not need to be calculated accurately.

The mice which, from their body weights and the appearance of the adrenal cortex, were evidently in the recovery stage showed a high uptake of iodine in the thyroids (Appendix B, Fig. 42). There were, unfortunately, very few mice injected which could be included in the "failure" group because very few survived for the necessary 7 days. There were, however, three typical cases, 2 males and 1 female, and the radio-active iodine concentration in the thyroids of these mice was extremely low (Appendix B, Fig. 43).

The thyroid glands of the 5-week-old mice showed, as was to be expected after 48 hours' exposure, a uniformly high uptake of the iodine. Those of the controls showed a mixture of high and low concentrations of iodine, corresponding exactly with the active and resting follicles seen in the ordinary preparations (Appendix B, Figs 44, 45).

These results confirm that the thyroid glands with flattened follicular epithelia are very inactive.

(e)/

(e) A summary of the changes observed in young mice after sudden exposure to cold.

There were no deaths among the 12- or 5-week-old A strain mice transferred from 21°C to -3°C but 23 per cent of the GFF mice and 48 per cent of the C57BL mice died before the end of 21 days' exposure. The 5-week-old A strain mice born in the cold all survived after they were placed alone in a cage. Only strain A mice were studied at the age of 3 weeks and there was a high mortality among those transferred from the warm to the cold room, 44 per cent dying before the end of 7 days' exposure. The 3-week-old mice born in the cold showed a mortality of only 20 per cent, less than half that of those transferred from 21°C.

All the mice transferred to the cold from 21°C suffered an initial loss of weight, probably due to loss of body fat. The weights of the 12-week-old mice tended to remain at this low level but the 5-week-old A strain mice and the surviving GFF mice started to grow again after 48 hours' exposure and by 21 days they were heavier than at the beginning of the experiment. The 3-week-old mice reared in the warm environment fell into two distinct groups after 48 hours' exposure. One group (group b) appeared to recover and had regained the initial loss of body weight by 7 days; the other group (group a) continued to lose weight and seemed to be slowly dying.

Among/

Among the mice reared at -3°C, those aged 5 weeks showed no loss of body weight at all, they continued to grow at their normal rate throughout the experimental period; most of those aged 3 weeks grew rapidly, although growth was checked in a few.

In all the mice transferred to -3°C from the warm room the adrenal glands increased in weight relative to body weight and the thymus glands decreased during the experiment. The sudden and premature atrophy of the thymus glands in these young mice is undoubtedly an index of an increased output of hormones from the adrenal cortex, since it is well known that injections of adrenal cortical extracts into a normal mouse results in an involution of the thymus. In the present experiments it was a frequent occurrence for the thymus to show a marked but transient increase in weight immediately after exposure, although no explanation can be given for this phenomenon. The change in weight of the adrenal and thymus glands in the GFF mice occurred rather more quickly after exposure than in the A strain. The hypertrophy of the adrenal glands and involution of the thymus was very pronounced in the 3-week-old "failure" group (a) mice. The relative organ weights of the mice reared in the cold did not alter signifi-:cantly during the experiment.

The histology of the adrenal and thyroid glands was affected/

affected by the sudden exposure of the mice to cold. In all those transferred from the warm room there was a depletion of lipid material, presumably cholesterol, from the zona fasciculata of the adrenal cortex immediately after exposure. In all except the 3-week-old "failure" group mice this lipid began to re-accumulate in the cortex after 48 hours' exposure and by 7 days the adrenals were in the state described by Selye as the "recovery" stage. The adrenals of the "failure" mice were found to be very severely depleted after 7 days' exposure. The 5-week-old mice born at $-3^{\circ}C$ showed no depletion of the adrenal cortex at any time during the experiment. The 3-week-old $-3^{\circ}C$ control mice showed the typical "alarm" reaction (depletion of lipid from the zona fasciculata) immediately after exposure but by 7 days all but one were in the "recovery" stage. The thyroid glands became more active immediately after exposure: the follicular epithelium was heightened, the nuclear volume increased and the colloid became less basic. In all the mice except the 3-week-old "failure" group this increased activity persisted throughout the experimental period. In the "failure" mice the activity progressively diminished after 12 to 24 hours' exposure and by 7 days the thyroid glands appeared to be very inactive; the follicular epithelium was flattened, the nuclei ovoid and the colloid very hard and basic. The histological appearance of activity or inactivity in the thyroid was confirmed by experiments involving the use of radio-active iodine.

The/

The changes in the thyroid, adrenal and thymus glands may be usefully further summarized in tabular form. A normal thyroid gland, that is the gland of a control mouse kept at 21°C, consists of a mixture of active and inactive follicles. In the Table below, therefore, both "active" and "hyperactive" imply degrees of activity above the normal. The term "recovery" applied to the adrenal glands implies some hyper-: trophy together with a return of lipid material to the zona fasciculata.

Table 8

Changes in endocrine glands after 7 days' exposure

Strain	Age in weeks	Thyroid	Adrenal	Thymus
A	12	hyperactive	recovery	reduced
A	5	hyperactive	recovery	reduced
GFF	5	hyperactive	recovery	reduced
A	5 (-3 C)	active	unchanged	unchanged
A	3 (failure)	very inactive	severely depleted & hypertrophied	very reduced
A	3 (survival)	hyperactive	recovery	reduced
A	3 (-3 C)	hyperactive	recovery	unchanged

(f) Effects of injecting ACTH or cortisone into mice kept at 21°C

Many workers (as discussed below, Section V) have asserted that the administration of cortisone depresses activity in the thyroid. In all the mice in which inactive thyroid glands were found, the adrenal and thymus glands showed indications of considerable cortisone secretion, namely increased adrenal size and reduction of the thymus. The possibility therefore arises that the lowered activity in the thyroid glands was primarily due to the high concentration of adrenal cortical hormones in the blood. To test this possibility 3-week-old mice reared at 21°C were injected subcutaneously with various doses of either ACTH or cortisone. The ACTH (ACTHAR GEL. 20 mg./ml. Amour Laboratories) was administered over a period of 7 days, one injection each day, and the cortisone (cortisone acetate 25 mg./ml. Roussell) was given as daily injections for 4 days. The solutions were diluted with distilled water and all the injections were of 0.1 ml.. The doses were decided partly from previous personal experience with albino rats and partly from data in the literature.

Some of the mice injected with ACTH were killed 24 hours after the last injection. The body and organ weights of these mice are given in Appendix A, Table 13.

The growth of the mice was unaffected by the injections of 0.25 to 1.5 mg. ACTH per day. The thymus weights of the males were/

were reduced to levels similar to those of the "failure" group of mice exposed to cold, that is to about 75 mg./100 g.: the thymus weights of the females, however, were only reduced to about 80 mg./100 g. compared with a mean thymus weight in the "failure" group of 39.8 mg./100 g. (Appendix B, Figs 48, 49). In both sexes the adrenal hypertrophy was less than that found in the "failure" groups. The thyroid glands of these mice were indistinguishable from those of the control mice and, owing to the length of time between the last injection and death, very little depletion of the adrenal cortex was observed.

In order to exclude the possibility that the thyroid glands, as well as the adrenals, had reverted to a normal state before the mice were killed, a further series of injections was carried out. Larger doses of ACTH were given and the mice were killed 6 hours after the last injection.

The body and organ weights are given below in Table 9.

Table 9/

Table 9

Sex	Total dose in 7 days mg. ACTH	Initial wt. g.	Final wt. g.	Thymus wt. mg./100 g.	Adrenal wt. mg./100 g.
♂	-	12.5	16.5	283.0	7.3
♂	7	13.0	18.0	73.3	7.8
♂	14	13.0	16.5	34.5	9.7
♂	14	9.5	13.5	28.9	10.4
♂	21	10.5	14.5	21.4	9.7
♀	7	11.0	13.5	67.4	10.4
♀	7	9.5	13.0	-	10.0
♀	14	11.5	14.5	41.4	13.8
♀	21	13.0	14.5	35.2	16.6
♀	21	10.0	13.0	32.3	11.5
♀	-	10.0	12.5	218.0	8.0

The growth and organ weights of the mice injected with 7 mg. ACTH were well within the limits of standard error calculated from the previous experiment (Appendix A, Table 13). Litter-mates of these mice which received 14 or 21 mg. ACTH grew at the normal rate, but their thymus weights were reduced to levels comparable with, or below, those of the mice of the "failure" group. Their adrenals were enlarged to an extent comparable with those of the "failure" group mice exposed for 7 days.

The/

The adrenal cortex in each of the injected mice was found to be heavily depleted, especially in those which received 14 or 21 mg. ACTH. The thyroid glands, on the other hand, were histologically indistinguishable from one another and from those of the controls.

The mice injected with cortisone were killed 6 hours after the last injection. The growth and organ weights of these mice are given below in Table 10.

Table 10

Sex	No. of mice	Total dose in 4 days mg. cortisone	Mean wt. change g.	Mean thymus wt. mg./100 g.	Mean adrenal wt. mg./100 g.
♂	3	-	+2.2	181.5	5.8
♂	1	2.5	+1.5	42.0	6.0
♂	3	5.0	+1.5	18.7	4.6
♂	3	10.0	+0.2	23.6	3.9
♀	4	-	+1.9	258.5	8.7
♀	3	2.5	0.0	28.3	7.2
♀	5	5.0	+0.7	23.4	6.5
♀	1	10.0	-1.0	30.4	4.4

The growth of these mice was retarded by the cortisone and both the thymus and adrenal weights were very much reduced with the/

the larger doses. The adrenal atrophy was found on inspection to have occurred mainly in the zona fasciculata. This zone stained densely with the Sudan stains.

Histologically the thyroids were quite normal and it was impossible to distinguish between those of the injected mice and those of the controls by their appearance (Appendix B, Figs 50-52).

The effects of ACTH in 7 days or of cortisone in 14 days were therefore far less than those found in some of the mice exposed to cold for only 48 hours.

(g) Effects of injecting ACTH or thyroxine before and during exposure.

There are several references in the literature (discussed below in Section V) to the effects of treatment with adrenal cortical and thyroid hormones on the survival of mammals at low temperatures. A series of experiments was therefore devised in order to determine whether these hormones produced any beneficial effects on the young mice used in the present experiments.

3-week-old A strain mice were transferred from 21°C to the cold room in the usual way but in addition some of them were given daily injections of either ACTH or thyroxine. The injections were given as 0.5 mg. ACTH in 0.1 ml. per day and 0.07 mg./

0.07 mg. thyroxine (l-thyroxine sodium salt, British Drug Houses Ltd.) in 0.2 ml. per day. As before, the solutions were made with distilled water. All the mice had their first injection 6 hours before transfer to -3°C.

The growth or time of death of mice receiving ACTH and of their litter-mate controls, all exposed to cold for 7 days, is given below in Table 11.

Table 11

0.5 mg. ACTH/day				Controls (not injected)			
Sex	Initial wt. g.	Final wt. g.	Died	Sex	Initial wt. g.	Final wt. g.	Died
♂	13		2nd day	♂	9		2nd day
♂	8		1st day	♂	11		1st day
♂	15.5	15	-	♂	15.5	17.5	-
♂	11	13.5	-	♂	15.5	17.5	-
♂	13.5	13.5	-	♂	12	10.5	-
♀	9.5		1st day	♀	12		2nd day
♀	8.5		2nd day	♀	9		2nd day
♀	9.5		2nd day	♀	9		4th day
♀	9		4th day	♀	11	11.5	-
♀	11		4th day	♀	9	9.5	-
♀	11.5	11.5	-	♀	11	11.5	-
♀	10.5	12.5	-	♀	12.5	13	-
♀	12	11	-	♀	11	11.5	-
				♀	14	14	-

Of the 13 injected mice 7 died, and of the 14 control mice 5 died, before the end of 7 days' exposure.

Other 3-week-old mice were each given a single large dose of 1.5 mg. ACTH 6 hours before exposure and their survival time at -3°C was noted. The results of this experiment are given in Table 12.

Table 12

Pre-dosage with 1.5 mg. ACTH			
Sex	Initial body wt. g.	Final body wt. g.	Died
♂	12		2nd day
♂	11.5		3rd day
♂	12.5		3rd day
♂	10		2nd day
♂	11.5		3rd day
♂	12.5	12	-
♂	12.5	11	-
♀	7		1st day
♀	7.5		1st day
♀	12	14	-
♀	11	12	-
♀	12.5	13	-

Injections/

Injections of ACTH either before or during exposure evidently offered no protection against cold in these young mice.

A few litter-mates of the mice receiving thyroxine were kept at 21°C and also received 0.07 mg. thyroxine per day. Table 13 gives the growth of these mice together with data for the growth and death of the injected and non-injected mice exposed to cold.

Table 13

0.07 mg. thyroxine/day				Controls (not injected)			
Sex	Initial wt. g.	Final wt. g.	Died	Sex	Initial wt. g.	Final wt. g.	Died
♂	11.5		2nd day	♂	9.5		2nd day
♂	10		2nd day	♂	12.5	13.5	-
♂	10.5		1st day				
♂	10.5		1st day				
♂	9	11	-				
♀	11		2nd day	♀	11		2nd day
♀	12		4th day	♀	10.5		5th day
♀	9		5th day	♀	10		5th day
♀	10.5		1st day	♀	9	12.5	-
♀	11		2nd day	♀	11	12	-
♀	9	11	-	♀	10.5	11.5	-
♀	9.5	13	-				
♀	10.5	13	-				
				(0.07 mg. thyroxine/day 21°C)			
				♀	11	15	-
				♀	9	15	-
				♀	9.5	13.5	-
				♀	8.5	12	-

Of the 13 injected mice 9 died, and of the 8 controls 4 died before the end of 7 days' exposure. The higher percentage of deaths in the injected group is not significant. The growth of the mice kept at 21°C was not retarded by the thyroxine, indicating that the dose given was not toxic.

These results give no evidence that treatment with ACTH or thyroxine confers an increased resistance to cold on 3-week-old A strain mice.

V. DISCUSSION

In the main, the sudden exposure to cold of young mice caused the expected results: adrenal depletion and hypertrophy, reduction of the thymus, increased thyroid activity and loss of body fat. The division of the 3-week-old mice into two distinct groups was not expected and the interpretation of this result presents some difficulty. Any attempt to interpret the reactions of the mice depends, in part, upon the correct assess-:ment of adrenal and thyroid activity from the data available in the present experiments. The first part of this discussion will therefore be concerned with this point.

(a) Assessment of criteria used for estimation of endocrine activity.

(i) Estimation of adrenal activity.

a. Thymus weight. In both the control mice and those that had been exposed to cold, the thymus weights were exceedingly variable. Rankin (1954) also found that the normal range of variation in thymus weight of adolescent mice was quite wide. She showed that during this period there were frequent slight involutions and regenerations of the thymus and that this continued into early maturity. (This may explain the capacity of the thymus glands of the 5-week-old female mice to regenerate although it does not suggest the reason for it.)

Rankin/

Rankin further considered that caution was advisable in assessing the results of dietetic and hormonal experiments involving thymic involution. Ingle and Li (1952), however, considered that the involution of the thymus is a more accurate index of ACTH secretion, and therefore of adrenal activity, than is adrenal hypertrophy or ascorbic acid depletion. In the present experiments described in Section IV involving the injection of ACTH into 3-week-old mice kept at 21°C, only the lowest dose level failed to produce a statistically significant decrease in thymus weight. In addition, the standard deviations of the mean thymus weight of each group of injected mice was small. It was only in the mice receiving the largest dose of ACTH that the adrenal weight increased much above that of the controls and the difference was only significant in the males. These results support the view that reduction of thymus weight is quite a sensitive index of adrenal cortical activity.

b. Ascorbic acid. Ascorbic acid has not been studied in the present experiments but since much work has been published on its effect on adrenal activity and on ascorbic acid content as an index of the secretory state of the cortex, the subject will be briefly discussed here. There is some evidence that the adrenal responses to cold can be modified to some extent by means of ascorbic acid. Dugal and Therien (1949), using white rats and guinea-pigs, showed that adrenal hypertrophy after cold/

cold exposure could be prevented by the administration of ascorbic acid. Ingle (1944) showed that injections of adrenal extracts or 11-oxy steroids increased the resistance of rats to the "stress" of the muscle-work test and Booker et al. (1951, 1955) found that injections of ascorbic acid would also increase the survival time of mice exposed to cold. In addition, the latter authors showed that the combined treatment of adrenalectomised mice with ascorbic acid and cortisone afforded greater protection than the steroid given alone. The function of ascorbic acid in these responses is, in the opinion of Dugal (1954), to potentiate the action of ACTH on the adrenal cortex.

Most of the evidence, then, indicates that the presence of ascorbic acid leads to an increased efficiency of adrenal cortical function. Knobil and Fregly (1955), however, found that ascorbic acid failed to influence either the adrenal weight changes or the alteration in adrenal cholesterol concen-: tration in rats exposed to cold for 24 hours. No explanation can be found for this discrepancy and it seems that the action of ascorbic acid may be variable.

It appears, therefore, that estimation of the ascorbic acid content alone is not an infallible criterion on which to base an assessment of adrenal activity. As mentioned above, Ingle and Li (1952) consider the degree of thymic involution to be a more reliable indication.

c./

c. Sudanophilia. Some of the experiments described in Section 4 indicate that the sudanophilic substance in the zona fasciculata is cholesterol. It is now necessary to consider whether the secretory activity of an adrenal gland can be held to be reduced when the zona fasciculata is depleted of cholesterol.

Hechter et al. (1951) and Haines (1952) showed that the adrenal cortex possesses enzyme systems which catalyse C-11, C-17 and C-21 hydroxylation of the steroid nucleus. Corticosterone and 17-hydroxycorticosterone appear to be the main steroids formed. Studies with acetate and cholesterol labelled with C_{14} indicate that these adrenal steroids may be derived from either acetate or cholesterol and that cholesterol is not an obligatory intermediate in corticosteroid synthesis from acetate. Conversely, cholesterol is not degraded to acetate before synthesis to corticosteroids. These results indicate that the depletion of cholesterol from the zona fasciculata does not preclude the possibility that synthesis of corticosteroids is still going on.

The studies of Popjak (1944) showed that there was not a complete parallelism between sudanophilia and what were considered to be the more specific ketosteroid reactions. In fact, he found that the relative amounts of sudanophilic material and ketones in the zona fasciculata were inversely proportional/

proportional. He reported that 24 hours after injury the depletion of sudanophilic and birefringent lipids from the zona fasciculata was associated with both an increase in the intensity of the phenylhydrazine reaction (supposedly showing ketosteroids) and a widening of the zone showing the reaction. By chemical estimation he found a reduction in cholesterol during the same period. More recent work, however, has thrown some doubt on the specificity of the phenylhydrazine and Ashbel-Seligman reactions for ketosteroids. Karnovsky and Deane (1954) found that the lipid droplets in fresh rat adrenals showed no histochemical carbonyl reaction by the Schiff or Ashbel-Seligman tests, whereas they became intensely reactive following several days' fixation in 10 per cent formalin. This result would suggest that the positive phenylhydrazine reaction found by Popjak could have been caused by the fixative. On the other hand, in the adrenal of a normal rat, presumably such as those tested by Karnovsky and Deane, the cholesterol content is high and, since the gland is not normally extremely active, the ketosteroid content may be expected to be very low. Wolman and Greco (1952) also consider that formaldehyde com-:bines with unsaturated lipids at the double bond. The resul-:ting complex contains a free carbonyl group which probably originates from the formaldehyde and this reaction product may be shown up by the Schiff reagent and by the Ashbel-Seligman procedure. Wolman and Greco therefore agree with Karnovsky and/

and Deane in that they consider that a positive Ashbel-Seligman reaction may be the result of oxidation of unsaturated compounds such as fatty acids. Wolman and Greco, however, do not rule out the possibility that a positive reaction may also indicate the presence of true detosteroids. This would explain the increase in intensity of the phenylhydrazine reaction above the normal found by Popjak after injury. At such a time the synthesis of corticosteroids would be expected to increase.

Lastly, it seems unjustifiable to assume that the areas of depletion in the fasciculata of the 12- and 5-week-old A strain mice (which, although not so extensive as those found in the dead or dying 3-week-old mice, were yet very large) represented areas in which no secretory activity was taking place. All the evidence in the literature would suggest that at this stage the secretory activity of the adrenal cortex is greatly increased.

Some doubt, therefore, is thrown on the classification of a severely depleted and hypertrophied fasciculata (such as is found in the 3-week-old "failure" mice) as an "exhaustion" phase, if this term implies that the rate of secretion of steroid hormones is greatly reduced. Although this phase frequently occurs at the point of death it may be indicative of a hyperactive gland.

In the light of the evidence available, it has been assumed, for/

for the purposes of the present experiments, that the estimation of adrenal activity from the degree of involution of the thymus, depletion of the zona fasciculata, and to a smaller extent by the adrenal hypertrophy, is justifiable.

(ii) Estimation of thyroid activity. Assessments of thyroid activity in the experiments described in Section IV have been made, for the most part, by inspection of the follicular cell height, the nuclear volume and the state of the colloid. The experiments involving auto-radiography showed that a histological appearance of activity of the thyroid gland or of individual follicles was accompanied by a high uptake of radio-active iodine; correspondingly, a histological appearance of inactivity was accompanied by a low uptake of I^{131}.

There is a possibility that some error may have been introduced among the females by a variation of thyroid activity during the oestrous cycle. Soliman and Reineke (1954) observed a maximum uptake of I^{131} from mice killed during pro-oestrus but found no significant alterations of body or thyroid weights during the four stages of the cycle. This cyclic change in activity would not, however, occur in the 3-week-old mice which were sexually immature and the thyroid activity of the 12- and 5-week-old females after exposure to cold was apparently the same as that of the males in the same conditions. Hurst and Turner (1947) have, in fact, shown that there is no sex difference in the thyroid secretion rate of 5-week-old mice, although adult females secrete twice as much thyroxine as males.

Tala (1953) compared the epithelial height with the nuclear volume as indicators of thyroid activity. He used young male guinea-pigs in which the thyroid glands were stimulated by small amounts of TSH and found that both methods were accurate to 0.00001 J.S. units of thyrotrophic hormone. Botkin et al. (1954) considered that the primary effect of TSH was to induce the release of hormone from the thyroid gland and Uhlenhuth et al. (1945) stated that during a rapid release of stored colloid there was a rise in follicular cell height. From his studies on the thyroid gland of the salamander Uhlenhuth concluded that large cells are a prerequisite for colloid release, although both high and low follicular cells are capable of elaborating and storing colloid in the follicles.

These results strongly suggest, though do not finally prove, that a histological appearance of activity reflects a high rate of discharge of thyroid hormone. (This does not apply, of course, after treatment with blocking agents such as thiouracil.) They should be considered in conjunction with other evidence, such as increases in metabolic rate and heat production. Taking into account all the evidence available, it has been assumed for the purposes of the present experiments that assessments of thyroid activity from the nuclear shape, state of the colloid and follicular cell height are justifiable.

(b)/

(b) Relationship between thyroid and adrenal activity.

The question next arises as to whether the peculiar response of the 3-week-old "failure" mice, namely the inactive thyroids and hyperactive adrenal glands after 7 days' exposure, could be the result of the interaction of these two glands. In other words, could the hyperactive adrenals be the direct cause of the inactive thyroids and vice versa? There is extensive evidence in the literature of a close relationship between thyroid and adrenal activity and this is best reviewed in two parts.

(i) Effects of adrenal cortical hormones on the thyroid gland. There is considerable disagreement about the effects of cortisone and ACTH on the thyroid gland.

Migeon et al. (1952) and Heinbecker and O'Neal (1953) state that cortisone has no effect on the thyroids of normal rats. O'Neal (1953) showed that the administration of cortisone did not influence the rate or volume of discharge of thyroxine into the blood in dogs and that the rate of destruction of the thyroxine was also not affected. Similarly, Engstrom and Markhardt (1954) found no evidence in man of an altered rate of destruction of thyroid hormone by cortisone. Again, Kuhl and Ziff (1952) observed no significant changes in the basal metabolic rate of patients receiving ACTH or cortisone and Bastenie, Gepts and Desclin (1952) state that cortisone never depresses the thyroid.

On/

On the other hand, many workers, including D'Angelo (1951), Botkin et al. (1952) and Brown-Grant et al. (1953), have reported that thyroid activity diminished following various "stresses" such as starvation or X-irradiation. They suggest that this was due to a reduced secretion of TSH. Bagorich and Timiras (1951) showed that a severe stress caused a decreased uptake of radio-iodine by the thyroid as well as a suppression of thyrotrophic stimulation.

These latter effects can sometimes be reproduced by treatment with ACTH or cortisone. Money et al. (1951) showed that both ACTH and cortisone decreased the thyroidal collection of I^{131} in the rat but stated that no effect was seen on the height of the follicular cells. Brown-Grant (1955) found that cortisone inhibited the release of thyroidal radio-iodine.

Further disagreement exists regarding the mechanism of this reported inhibitory influence of ACTH or cortisone on the thyroid gland.

Albert, Tenney and Ford (1952) state that ACTH, while lowering the 24-hour accumulation, does not affect the discharge of thyroidal I^{131}. They conclude, therefore, that it has no effect on the production of TSH. Paschkis et al. (1952) report that cortical hormones act directly, and not necessarily through pituitary suppression, in inhibiting the TSH-thyroid mechanism, since this action can be produced in hypophysectomised/

hypophysectomised rats. Similarly, Woodbury, Gosh and Sayers (1951) found that ACTH or cortisone inhibited the action of TSH when given with TSH to hypophysectomised rats. Shellabarger (1954), working with White Leghorn cockerels, found that cortisone inhibited thyroidal uptake in the presence or absence of TSH and so concluded that it had a direct action on the thyroid in the domestic fowl. Lastly, the work of Lederar (1952) also indicates a direct action of cortisone on the thyroid gland of the rat. He showed that the increase in weight of the thyroids by stimulation with exogenous TSH was inhibited by cortisone, although it did not affect the weight or histological changes caused by propyl thiouracil.

On the other hand, several workers have found that cortisone affects thyroid secretion indirectly through the pituitary. Hill et al. (1950), from clinical evidence, and Brown-Grant, Harris and Reichlin (1954b), working on the rabbit, state that cortisone and ACTH act mainly by suppressing pituitary TSH secretion. It seems doubtful whether ACTH itself has any direct effect on the thyroid, since Brown, Woodbury and Sayers (1952) have shown that adrenalectomy abolishes the inhibition of TSH action when given concurrently with ACTH in hypophysectomised rats.

It therefore appears that most of the investigators who find that cortisone or ACTH depresses thyroid activity are of the/

the opinion that the inhibition takes place either by a suppression of TSH secretion in the pituitary gland or by direct action on the thyroid gland itself. Two other hypotheses have been put forward. First, Goldenberg et al. (1955) consider that in man the site of hormonal interaction is at the cellular metabolic level. Secondly, Badrick et al. (1954) showed that conditions of "stress" such as extremes of temperature or electric shock, led to only a transitory reduction of I^{131} uptake by the thyroid. It was also independent of the anterior pituitary or the adrenal since hypophysectomised or adrenal-:ectomised rats showed the same degree of inhibition as normal ones. Badrick and his colleagues consider that this inhibition of thyroid function is due to the release of vaso-constrictor substances into the blood when the animal is stressed.

There have been several theories put forward to explain the variety of reported effects of cortisone and "stress" on the thyroid gland.

Ingbar (1953) suggested that the decreased I^{131} uptake by the thyroids after cortisone treatment could be explained by the increased iodine excretion from the kidneys caused by cortisone. Greenspan, Gifford and Deming (1953) pointed out that the method of administration may also affect the results produced. They showed that some cortisone effects require sustained levels of cortical hormones in the blood stream and these are produced only by subcutaneous injections.

Subcutaneous/

Subcutaneous injections of cortisone led to loss of body weight and atrophy of the thymus and adrenal, whereas intraperitoneal injections did not. This may be the reason for some, although not all, of the conflicting reports on the effect of cortisone on thyroid activity.

The conflicting results reported by Aterman and Greenburg may also be explained in the light of more recent work. In 1953 Aterman and Greenburg showed that high dose levels of cortisone resulted in exophthalmos and thyroid enlargement in young rats and presumably acted as a mild goitrogen. In 1954, on the other hand, they found that prolonged cortisone administration to hypophysectomised adult rats produced no ocular protrusion. These results would suggest an indirect action on the thyroid via the pituitary, resulting in a hyperactive gland. Boas and Scow (1954), however, found that young rats dwarfed by thyroidectomy or cortisone treatment develop an apparent exophthalmos. They stated that this was due to normal eyeball growth in the presence of a marked inhibition of head and body growth. Williams (1955) also showed that cortisone-induced exophthalmos in young guinea-pigs was due to retarded growth of the skull and not to a direct effect on the thyroid. Whether exophthalmos develops after cortisone administration evidently depends upon the age of the experimental animal.

The influence of cortisone on thyroid function is thus seen/

seen to be very variable. In the present experiments, the effects of injections of cortisone or of ACTH on 3-week-old A strain mice were far from dramatic. As stated above, there was no change visible on careful inspection; actual measurement of the height of the epithelial cells might have revealed a difference but no such alteration could have compared in extent with the effect produced by cold exposure in mice of the "failure" group.

It is possible to state, therefore, that whatever may be the primary factor or factors in the production of the hypoactive condition of the thyroid glands, it is not cortisone acting alone. There is a possibility that a fall in body temperature is responsible, since Verzar et al. (1953) have shown that in rats a lowering of the body temperature to 15°C to 20°C is accompanied by complete inactivity of the thyroid gland.

(ii) Effects of thyroid hormone on the adrenal gland. The evidence for the influence of thyroid hormone on the adrenal gland is more straightforward.

Deane and Greep (1947), Zarrow and Money (1949), Maqsood (1950) and Freedman and Gordon (1950) showed that hypothyroidism resulting from surgical thyroidectomy or the administration of anti-thyroid drugs was accompanied by adrenal cortical atrophy. The activity of such an atrophied gland is not, however, necessarily/

necessarily reduced since it may be capable of a greater than normal rate of secretion. Finerty and Hess (1951) found that the depletion of ascorbic acid from the adrenals after a scald was greater in thyroidectomised rats than in controls. Freedman and Gordon (1955) exposed hypothyroid rats to cold and they too observed a response greater than normal.

Zarrow and Zarrow (1951) attempt an explanation of the cortical atrophy by suggesting that in the hypothyroid rat the pituitary is secreting an exceptionally large amount of thyro-:trophic hormone and that consequently less ACTH is synthesised. Halmi and Bogdanove (1951), on the other hand, found no signi-:ficant difference in ACTH content between normal and thyroidectomised rat pituitaries. They believe therefore that the reduction in ACTH output is not primarily due to an inability of the pituitary to synthesise ACTH.

Many authors, including Gardener (1942), Deane and Greep (1947) and Maqsood (1954) have reported that injection or feeding with thyroxine results in adrenal hypertrophy. Lowenstein and Zwemer (1942), however, report that with very high doses of thyroid hormone, involution of the adrenal cortex occurs. It is doubtful whether the mechanism of adrenal enlargement is directly related to an elevated basal metabolic rate, since Mercier-Parot et al. (1951) have shown that enlargement is not produced with dinitrophenol. It is more likely that, as suggested by Tepperman, Engel and Long (1943a, b/

b), treatment with thyroid hormone is only one of many "stressful" agents producing adrenal hypertrophy. This effect depends on the presence of an intact pituitary gland, since Mercier-Parot et al. (1951) found that it did not occur in hypophysectomised rats.

There is no evidence in the literature that hypertrophy of the adrenal gland, such as was found in the 3-week-old "failure" mice, is ever the result of an inactive thyroid gland. The very high rate of secretion in the adrenal cortex of these mice may, however, be partly the result of a lowered rate of thyroid secretion since, as already mentioned, hypothyroid mammals exposed to cold may show a greater than normal adrenal response.

(c) Mechanisms of control of adrenal and thyroid activity.

The hyperactive state of the adrenal glands in some of the 3-week-old mice may be partly the result of the inactive thyroid glands. However, the reverse is not true: that is, inactivity of the thyroid glands cannot itself be caused by a high concentration of adrenal cortical hormones in the blood. In order to determine the possible reasons for thyroid inactivity at a time when hyperactivity would be expected and for the continued high rate of secretion in the adrenal glands (that is, no apparent "recovery" stage), it is necessary to consider the mechanisms which control the activity of these two glands.

(i)/

(i) Control of thyroid activity. There is extensive evidence that exposure to cold leads to:

(a) production of TSH by the anterior pituitary;

(b) increased activity of the thyroid gland;

(c) increased utilisation of thyroid hormone by the body tissues.

The first possibility is that exposure to cold results in an increased production of TSH and that this in turn leads to an increased rate of secretion of thyroid hormone with a rise in its concentration in the blood. This would finally cause a higher rate of tissue metabolism. If the initial response of the animal is an increase in TSH production, it seems probable that this could be elicited by means of nervous stimu-:lation. Brown-Grant et al. (1954a) produced evidence to support the view that the central nervous system can influence thyroid activity through alterations in secretion of TSH from the anterior pituitary. Brolin (1945) also suggested that the release of thyrotrophic hormone from the pituitary glands of rats exposed to cold was controlled by a neural mechanism.

The other possible mechanism, considered by Rand et al. (1952) to be the more likely, is an initial increase in the rate of utilisation of the thyroid hormone by the body tissues. This would, it is supposed, lead to a lowering of the concen-:tration of thyroid hormone in the blood and a compensatory increase in the secretion of thyrotrophic hormone and so to an increase/

increase of thyroid activity. Several workers have produced evidence supporting this theory. Dempsey and Searles (1943) found evidence of thyroid activity after section of the pituitary stalk. Uotila (1940) showed that although section of the pituitary stalk in rats exposed to cold prevented hypertrophy of the thyroid, it did not affect the hypertrophy of a thyroid remnant after subtotal thyroidectomy.

Barnett and Greep (1951), working with rats, showed that the hypophyseal blood vessels did not regenerate after section of the pituitary stalk. There was not only histological evidence of hypofunction of the pituitary gland but the adrenals and thyroid were atrophied, indicating a subnormal release of adrenocorticotrophic and thyrotrophic hormones. However, on exposure to cold there was an increase in output of both these hormones, resulting in adrenal hypertrophy and an increase in the follicular cell height of the thyroid glands. This suggests that the pituitary gland responded to the "stress" of exposure despite the absence of direct neural or vascular connections with the central nervous system. Barnett and Greep suggest that the titre of cortisone and thyroid hormone in the blood plays a major role in the regulation of pituitary adrenocortico-: trophic and thyrotrophic activity.

It appears therefore that the activity of the thyroid gland may be controlled by two methods, one neural and the other chemical.

(ii)/

(ii) Control of adrenal activity. It is unlikely that the primary cause of adrenal hypertrophy after cold exposure is the increased thyroid activity, especially since in the "failure" mice of the present experiments there was adrenal hypertrophy and depletion in the presence of inactive thyroid glands. Tepperman, Engel and Long (1943) have pointed out that adrenal cortical hypertrophy always depends on an increased amount of circulating ACTH. It has been postulated that stimulation of the pituitary gland in conditions of "stress" is in turn due to adrenalin. Gemzell et al. (1951) showed that adrenalin partly prevented the rise in plasma ACTH after adrenalectomy in rats but accentuated the fall in pituitary ACTH concentration. These authors concluded that adrenalin inhibited ACTH synthesis but stimulated ACTH secretion. Vogt (1951a, b) showed, however, that adrenalin is not the most important factor in evoking the response of the adrenal cortex of rats to emotional stimuli, since the response could be produced in demedullated animals. Guillemin (1955) confirmed these results and in addition showed that adrenalin was not responsible for stimulation of the pituitary after a systemic stress, such as injection of formalin.

It has already been mentioned that Barnett and Greep (1951) consider that the concentration of cortisone in the blood plays an important part in the regulation of pituitary adrenocortico-: trophic activity. Sayers and Sayers (1947) also attribute the/

the release of ACTH to a reduction in circulating cortical steroids and this reduction is held to depend in turn on tissue requirements. Evidence that ACTH secretion is sensitive to levels of circulating corticosteroids is given in the work of Gemzell et al. (1951) who showed that increased blood levels of ACTH occurred in rats after adrenalectomy. Farrell and Laqueur (1955), working with dogs, showed that the ACTH content of the pituitaries declined with the length of time of administration of cortisone.

Some workers have recently put forward the theory of a dual mechanism of ACTH control. Ganong and Hume (1955) found that destruction of part of the hypothalamus of dogs blocked the "stress-induced" increases in ACTH secretion but did not modify the depression in ACTH secretion following corticoid administration. Fortier (1951) and Anand et al. (1954) concluded that ACTH secretion in rats could be mediated through the hypothalamus by nervous stimuli as well as by a purely hormonal mechanism in response to systemic stimuli. In addition, Saffran and Schally (1955) found evidence that the posterior pituitary may activate the anterior pituitary to release ACTH and Martini and Morpurgo (1955) suggest the anti-diuretic hormone as a possible neurohumeral transmitting agent.

The function and control of the zona glomerulosa of the adrenal/

adrenal cortex constitute a separate problem and since this zone was not studied in detail in the present experiments the subject will not be discussed here.

The secretory activity of the zona fasciculata is thus seen to be controlled in a similar way to that of the thyroid gland, namely by both chemical and nervous mechanisms.

(d) Thyroid and adrenal cortical function in infant mammals.

It has been stated (Section IIIe(iv)) that rats and mice are poikilothermous at birth and begin to regulate their body temperature efficiently only at about 18 days of age. The evidence from adult mammals shows that one aspect of this regu-:lation is the ability of the adrenal, thyroid and pituitary glands to change their rates of secretion. The inference can be drawn, therefore, that this ability is only developed after birth. Research on the endocrinology of foetal and infant mammals is, however, not nearly as extensive as that on adults.

Jones et al. (1953) have shown that high levels of ACTH in the maternal blood of rats can enter the foetus and produce a stimulation of the foetal adrenal. Kitchell and Wells (1952) demonstrated that unilateral adrenalectomy of 20-day rat foetuses resulted in a compensatory hypertrophy of the remaining adrenal but that this hypertrophy could be prevented by implantation of cortisone pellets. They interpreted these results as indicating a functional reciprocal relation between the hypophysis and the adrenal of foetal rats. The experiments

of/

of Raynaud and Frilley (1950) indicate the secretion of a corticotrophic hormone by the hypophysis of foetal mice. Finally, Sethre and Wells (1951) showed that the growth of the thyroid of foetal rats can be accelerated and the follicular cell height increased by treatment with TSH.

It appears, therefore, that the adrenal cortex and the thyroid of rats and mice are both responsive to stimulation before birth.

Rinfret and Hane (1955) showed that extracts of pituitary tissue from 4- to 7-day-old rats were capable of reducing the adrenal ascorbic acid of hypophysectomised adult and of hypophysectomised 4- to 7-day-old rats. Thompson and Blount (1954) subjected 1- to 14-day-old mice to "stressing" by heat, adrenalin or injections of ACTH. Significant eosinopoenia after heat did not occur until about the 11th day and by the 14th day the adult pattern of eosinophil depletion was established. Holtkamp et al. (1949) studied the ability of newborn rats to stand exposure to cold with or without the administration of adrenal cortical extract. They found that adrenal cortical hormones offered no protection against cold in rats less than 16 days old but did so in older animals. These results suggest that adrenal cortical hormones protect rats against cold through a mechanism that is not developed before 16 days of age. Jailer (1951) in confirmation showed that the pituitary-adrenal system of young rats did not respond to any one "stress" until the animal was 18 days old.

Although there are several examples cited in the literature of both adrenal cortical and thyroid hormones increasing the resistance of mammals to cold, in the present experiments neither ACTH nor thyroxine gave any protection to the 3-week-old A strain mice which were suddenly exposed to cold. Deaths occurred in about the same proportion in both the injected and non-injected groups. The thyroxine-injected control animals which were kept at 21°C did not show the retardation of growth that was expected. However, Belasco and Murlin (1941) found that young rats were much more resistant to changes in body weight after treatment with thyroxine than were adults.

In mice of each age group, namely 3, 5 and 12 weeks, used in the experiments described in Section IV, the most critical time (inferred from the fact that most of the deaths occurred at this time) was between 24 and 72 hours after exposure. By 48 hours the difference between the "failure" and "survival" groups of 3-week-old mice was quite pronounced. 48 hours after exposure was also the time at which those aged 5 weeks began to grow again: evidently this was the minimum period required for the process of physiological adaptation to cold to begin. Fregly (1953), working with rats, found that acclimatisation to cold, as judged by the colonic cooling rate, occurred some time between the 2nd and 4th day of exposure to cold air (5°C). These results agree very well with the findings with mice given above.

VI. CONCLUSIONS

The absence of deaths among the 5-week-old A(V) and A(Y) mice after transfer to -3°C indicates that the process of adaptation to cold exposure is more efficient in these than in the C57BL or GFF strains. This indication is supported by the fact that there is a much greater reduction in fertility of the C57BL and GFF strains than of the A(V) strain after transfer to the cold as adults. The death rate of the 5-week-old C57BL mice suddenly exposed to cold was 48 per cent, but it was found that this strain could be bred indefinitely at the low tempera-: ture. On the other hand, while the death rate of the GFF mice under the same conditions was only 23 per cent, they could not be bred indefinitely. Fertility in the GFF mice was, however, lower than that of the other strains at 21°C (probably the result of a long period of dioestrus) and the reduction at -3°C must have lowered the fertility to a level at which the stock could not be maintained.

The fertility of the C57BL strain at -3°C, measured in terms of the number of young weaned per pair, was approximately the same as that of the A(Y) strain and slightly lower than that of the A(V) strain. The differences between the C57BL and A strains lie in the greater number of young born per litter in the C57BL mice and the larger proportion of whole litters that are lost in this strain. This is possibly a further indication of an inferior ability of the C57BL mice to "adapt" to the cold environment even during infancy.

A review of the evidence available in the literature indicates that the age at which the pituitary-adrenocortical systems of rats and mice can react to "stress" is 11 to 18 days. This is much the same as the age at which the control of body temperature begins (discussed in Section IIIe(iv)). It is therefore a reasonable hypothesis that the maturation of the pituitary-adrenal and pituitary-thyroid systems is important in the development of the control of body temperature.

It was found that the 3-week-old A strain mice could be divided into two groups of which one, the "survival" group, gave a response similar to that of adult mice while the other, the "failure" group, failed to adapt to the cold environment. These results suggest that in the A strain mice an important stage in the development of the control of body temperature is usually reached some time between the ages of 20 and 24 days. The fact that a single A strain mouse aged 5 weeks failed to enter the "recovery" stage after 7 days' exposure indicates that this change can be considerably delayed. In the GFF and C57BL mice the age of "maturation" is evidently much later and this may be associated with the difference between the growth of the C57BL and A strain mice reared at -3°C.

From the difference in mortality between the 3-week-old mice reared at -3°C and those of the same age reared at 21°C, it seems that, in general, the development of the adult type of response/

response to cold occurs earlier in mice subjected to a cold environment from birth. If the maturation process is delayed in mice living at -3°C they are quickly eliminated, so that at 5 weeks all the survivors are completely adapted. Although all the 5-week-old A strain mice born at 21°C survived the sudden exposure to cold, they did go through the phases of Selye's (1946) "general adaptation syndrome". By contrast, mice of the same age reared at -3°C showed no signs of distress at all when placed alone in a cage.

It was impossible to predict before the beginning of the experiment whether a 3-week-old mouse would survive the transfer from 21°C to -3°C or not. A larger proportion of heavier mice was found in the "survival" group but the scatter was such that size could be discounted as the most important factor. All the animals that died had been healthy, growing mice in which the endocrine system was apparently functioning perfectly normally at 21°C.

An examination of the animals of all ages which survived the experiment showed that the re-accumulation of sudanophilic material in the adrenal cortex occurred after 24 to 48 hours' exposure, while the increase in activity of the thyroid glands was maintained throughout the 21-day experimental period. It would seem, therefore, that for a complete recovery from exposure, or for adaptation to cold, the high rate of ACTH secretion began to decline after about 24 hours. On the other hand/

hand, the TSH output, and therefore thyroid activity, remained at a high level for a long time. However, the endocrinological picture of the dead or dying mice between 48 hours' and 7 days' exposure was very different; the adrenal glands were hyper-:active and the thyroid glands were very inactive.

This difference seems to be the main distinction between the juvenile and the adult type of reaction to cold. In the former there must be a failure of one of the mechanisms for increasing the metabolic rate and heat production which, with the loss of the early tolerance towards a low body temperature, results in a great and prolonged "alarm" reaction. In the adult the "alarm" reaction is relatively short and there is an immediate and lasting increase in heat production.

One of the mechanisms for increasing the metabolic rate and heat production is an increase in carbohydrate metabolism. As mentioned in Section IIIe(iv), it has been found that in rats effective thermo-regulation depends on the ability of the liver and muscles to release stored glycogen on exposure of the animal to cold, and that this ability does not develop until the age of 18 days. If a similar change takes place in mice, it seems likely that in the A strains it occurs at 20-24 days, since this is the age at which an important stage in the develop-:ment of the control of body temperature has been found to occur.

There/

There is a great deal of evidence that in adult mammals a rise in the blood concentration of thyroid hormone is accompanied by an increase in carbohydrate metabolism. If it is assumed that, in mice of all ages, the tissue demand for thyroid hormone is associated with carbohydrate metabolism, there are at least two possible explanations of the juvenile type of reaction to cold.

(1) The first involves pituitary function. If the pituitary failed to respond to the stimuli for an increased rate of elaboration and secretion of TSH, the thyroid would not be in turn stimulated to increased activity. This would mean that the stress of cold exposure would become more severe and the adrenal gland stimulated to even greater activity. Such a situation could become a vicious circle, the demand for adrenal cortical hormones becoming so high that the pituitary secretion of ACTH would take place to the detriment of TSH secretion. In other words, there would be a "shift" from TSH to ACTH production with an inevitable subsequent increase in the ill-effects of cold exposure.

If this explanation were correct, injections of ACTH or thyroxine should alleviate these effects. There was no evi-:dence in the present experiments, however, that either of these hormones had beneficial results.

(2) If, on the other hand, the major factor were not the response/

response of the pituitary gland but the tissue demand for thyroid hormone, the effects of exposure to cold and of injec-: tions of ACTH or thyroxine could be explained. It has been fairly well established that a major part of the control of pituitary TSH secretion is by the titre of thyroid hormone in the blood; this in turn depends upon tissue demand. If this demand were not increased on exposure to cold, the pituitary would not be stimulated to greater secretion of TSH. Injec-: tions of thyroxine would be useless under these circumstances and ACTH or adrenal cortical hormones would not assist the necessary increase in heat production. The treatment with thyroid hormone of animals in which the adult type of reaction had been developed, that is, in which there was a rise in carbohydrate metabolism and an increased demand of the tissues for thyroid hormone, would certainly result in an increased resistance to cold.

It has been fairly well established that the activity of the thyroid gland is controlled by two methods, one chemical and the other neural. The initial increase in TSH secretion appears to be by nervous stimulation. The long-term control, on the other hand, is probably almost entirely by the chemical method involving the blood concentration of thyroid hormone. If the control of pituitary thyrotrophic function normally takes place in two such stages, it is the second stage which, perhaps, fails/

fails to function in young mice. After a transient increase in activity the thyroid reacts like that of a poikilothermous animal and, with a failure of the tissues to mobilize their glycogen reserves, an involution of the glandular epithelium takes place when the body temperature begins to fall.

This hypothesis requires testing by further experiment, since it is based only on indirect evidence.

Appendix A.

Tables 1 - 14.

Table 1

Total growth. Males. (± = Stan

Strain	Temp.	Generation	3 weeks		5 weeks	
			No. of mice	Mean wt. g.	No. of mice	Mean wt. g.
A(V)	21°C	1	43	9.76 ± 0.16	37	17.39 ± 0.4
"	"	2 - 4	214	9.96 ± 0.12	54	17.47 ± 0.4
"	-3°C	1	42	7.95 ± 0.21	32	14.97 ± 0.7
"	"	2 - 3	74	8.78 ± 0.22	22	15.59 ± 0.7
A(Y)	21°C	1	49	10.13 ± 0.19	38	19.68 ± 0.3
"	"	2 - 3	270	10.59 ± 0.10	45	20.37 ± 0.3
"	-3°C	1	32	8.39 ± 0.24	24	16.46 ± 0.5
"	"	2 - 3	74	8.16 ± 0.16	33	15.86 ± 0.4
C57BL	21°C	1	43	7.97 ± 0.18	26	17.08 ± 0.5
"	"	2 - 4	136	8.48 ± 0.11	22	18.02 ± 0.5
"	-3°C	1	19	8.24 ± 0.37	11	14.64 ± 1.
"	"	2 - 3	23	7.09 ± 0.27	11	13.91 ± 1.

Table 2

Relative growth. (Standard deviation given in brackets)

Age	Strain	No. of mice		Body wt. g.		Body length mm.		Tail length mm.	
		21°C	-3°C	21°C	-3°C	21°C	-3°C	21°C	-3°C
Males									
3 weeks	A(V)	60	62	10.6(1.9)	8.6(1.8)	64.8(4.9)	57.9(4.2)	58.5(8.9)	46.4(8.9)
"	A(Y)	58	22	10.3(1.8)	8.2(1.2)	64.3(4.7)	57.1(3.2)	66.4(4.9)	52.3(3.2)
16 weeks	A(V)	22	11	33.5(4.4)	26.2(2.7)	94.3(6.3)	88.0(3.4)	74.7(13.2)	56.1(6.8)
"	A(Y)	15	10	32.4(5.0)	26.5(3.5)	96.9(2.4)	89.0(6.6)	93.7(1.8)	76.7(6.6)
"	C57BL	11	8	29.1(2.3)	28.1(3.4)	90.0(5.9)	91.0(5.6)	85.9(2.8)	65.1(4.0)
Females									
3 weeks	A(V)	49	72	10.1(1.8)	8.3(1.5)	63.5(4.7)	56.8(4.5)	60.2(8.1)	45.0(8.2)
"	A(Y)	61	29	10.2(1.4)	8.3(1.9)	64.4(3.3)	57.6(4.9)	66.9(3.9)	52.9(4.6)
16 weeks	A(V)	21	21	29.4(4.9)	23.6(2.0)	92.2(4.2)	85.4(4.7)	70.3(11.1)	62.9(9.9)
"	A(Y)	28	15	27.7(3.7)	24.5(2.8)	94.5(3.9)	90.9(3.7)	91.8(2.9)	78.7(5.4)

Table 3

Reproduction. (± = Standard e

	A(V)		A(
	21°C	-3°C	21°C
No. of pairs	14	7	15
Mean no. young born/pair	24.71 ± 1.75	17.43 ± 1.94	27.13 ± 2.96
Mean no. young weaned/pair	17.71 ± 1.65	6.14 ± 1.25	20.67 ± 2.50
Mean no. young born/litter	4.68 ± 0.21	4.52 ± 0.38	5.29 ± 0.20
Mean no. young weaned/litter	4.20 ± 0.23	3.91 ± 0.54	4.70 ± 0.22
Mean no. litters born/pair	5.36 ± 0.28	3.86 ± 0.42	5.13 ± 0.54
Mean no. litters weaned/pair	4.21 ± 0.25	1.57 ± 0.28	4.40 ± 0.52
No. of barren pairs	0	0	1
No. of pairs weaning no young	1	0	0
No. of litters of unknown no. of young	0	1	1

Table 4

Oestrus. Mice transferred to -3°C as adults

(Standard deviation given in brackets)

Strain	21°C (controls)		-3°C	
	No. of mice	No. of oestrous cycles in 4 weeks	No. of mice	No. of oestrous cycles in 4 weeks
A(V)	3	3.7(0.2)	7	3.0(0.8)
A(Y)	2	3.0(1.0)	5	2.0(0.6)
C57BL	4	3.5(0.5)	6	2.3(0.8)
GFF	2	2.0(0.0)	3	2.3(0.4)

Table 5

Oestrus. Mice reared at -3°C

(Standard deviation given in brackets)

Strain	21°C (controls)		-3°C	
	No. of mice	No. of oestrous cycles in 14 days	No. of mice	No. of oestrous cycles in 14 days
A(V)	6	2.7(0.7)	2	2.5(0.5)
A(Y)	4	3.0(0.0)	4	2.8(0.7)

Table 6

Unmated mice. Relative adrenal and thymu

16 weeks

(Standard deviation given

Strain	No. of mice		Body wt. g.		Adrenal wt. mg./100 g	
	21°C	-3°C	21°C	-3°C	21°C	-3°C
			Males			
A(V)	17	6	33.1(4.7)	26.8(2.7)	4.53(0.9)	10.02(3.6)
A(Y)	14	6	32.7(5.0)	26.3(3.7)	4.22(0.8)	6.56(2.3)
C57BL	13	7	28.9(2.2)	28.1(3.6)	5.86(1.8)	8.62(1.7)
			Females			
A(V)	16	11	29.2(5.3)	23.5(1.8)	9.58(1.3)	20.91(3.8)
A(Y)	19	9	27.1(3.6)	24.2(3.3)	10.22(2.7)	11.61(4.1)

* Indicates a significant differ

Table 7

12-week-old mice. (± = S

Time of exposure	Males			
	Mean initial body wt. = 28.3 ± 0.5 g.			
	No. of mice A(V) + A(Y)	Mean body wt. change g.	Mean adrenal wt. mg./100 g.	Mean thymus wt. mg./100 g.
0 hours (control)	2 + 3	-	4.60 ± 0.28	72.34 ± 3.7
6 hours	2 + 3	-0.5 ± 0.2	4.62 ± 0.49	78.10 ± 5.1
24 "	2 + 3	-0.8 ± 0.3	4.52 ± 0.40	64.52 ± 6.6
48 "	3 + 2	-2.5 ± 0.4	5.16 ± 0.09	65.77 ± 10.0
7 days	2 + 3	-3.0 ± 0.5	4.63 ± 0.54	38.06 ± 3.6*
21 "	2 + 3	-3.0 ± 0.9	5.49 ± 0.58*	30.32 ± 3.1*
21 days (control)	3 + 4	+1.0 ± 0.3	4.05 ± 0.15	51.55 ± 4.3

*Indicates a significant diffe

Table 8

5-week-old mice. Strain A. (± = Standar…

	Males				
	Mean initial body wt. = 19.0 ± 0.3 g.				
…ime of …xposure	No. of mice A(V) A(Y)	Mean body wt. change g.	Mean adrenal wt. mg./100 g.	Mean thymus wt. mg./100 g.	No. of mice A(V) A…
… hours (…ontrol)	3 + 2	-	6.88 ± 0.42	171.97 ± 10.9	2 + …
… hours	2 + 3	-1.1 ± 0.2	4.86 ± 0.46	174.78 ± 7.8	2 + …
"	2 + 3	-1.4 ± 0.2	5.73 ± 0.71	148.41 ± 1.0	1 + …
"	3 + 2	-1.6 ± 0.4	7.38 ± 0.59	144.63 ± 14.6	3 + …
… days	3 + 2	-0.5 ± 0.9	6.04 ± 0.95	89.06 ± 14.7*	2 + …
"	2 + 3	+1.2 ± 0.8	7.76 ± 1.06	63.23 ± 10.5*	1 + …
… days (…ontrol)	5 + 6	+2.1 ± 0.3	6.66 ± 0.22 (6.26 ± 0.20)	141.00 ± 7.7 (132.50 ± 7.5)	5 + …
… days (…ontrol)	2 + 3	+3.8 ± 0.8	5.96 ± 0.24 (5.60 ± 0.24)	111.10 ± 5.5 (104.5 ± 5.3)	3 + …

Brackets indicate unadjusted figures, i.e. no allowanc…

*Indicates a significant difference at P = 0.05.

+Indicates a significant difference using adjusted figu…

Table 9

5-week-old mice. Strain A, -3°C

Time of exposure	Males			
	Mean initial body wt. = 15.4 ± 0.9 g.			
	No. of mice A(V) + A(Y)	Mean body wt. change g.	Mean adrenal wt. mg./100 g.	Mean thymus wt. mg./100 g.
0 hours	2 + 2	-	7.50 ± 0.70	117.12 ± 7.27
48 hours	2 + 3	+0.6 ± 0.1	7.66 ± 0.39	147.67 ± 22.2
7 days	3 + 2	+1.6 ± 0.6	8.16 ± 0.17	111.00 ± 6.4
21 "	1 + 3	+3.9 ± 0.5	6.39 ± 0.13	79.08 ± 9.5

Table 10

5-week-old mice. Strain GFF. (±

Time of exposure	No. of mice	Mean body wt. change g.	Mean adrenal wt. mg./100 g.	Mean thymus wt. mg./100 g.	No. o mice
	Males				
	Mean initial body wt. = 20.4 ± 0.5 g.				
0 hours (control)	4	-	8.59 ± 0.62	172.9 ± 11.7	4
24 hours	6	-1.0 ± 0.2	9.12 ± 0.72	164.7 ± 10.9	6
48 "	5	-3.0 ± 0.4	8.13 ± 0.41	111.9 ± 9.6*	5
7 days	6	-0.3 ± 0.3	9.43 ± 0.98	111.5 ± 14.6*	5
21 "	5	+3.2 ± 0.6	8.22 ± 0.67	84.7 ± 14.5+	4
7 days (control)	5	+2.5 ± 0.4	8.07 ± 0.79 (7.35 ± 0.71)	211.5 ± 19.7 (192.6 ± 18.0)	5
21 days (control)	5	+4.2 ± 0.4	6.93 ± 0.20 (6.30 ± 0.20)	146.2 ± 16.4 (133.0 ± 15.0)	5

Brackets indicate unadjusted figures, i.e. no allo

*Indicates a significant difference at P = 0.05.

+Indicates a significant difference using adjusted

Table 11

3-week-old mice. Strain A. (± = S

Time of exposure	No. of mice A(V)	No. of mice A(Y)	Mean body wt. change g.	Mean adrenal wt. mg./100 g.	Mean thymus wt. mg./100 g.	No. mic A(V)
	Males					
	Mean initial body wt. of all mice = 11.1 ± 0.8 g.					Mean
	" " " " "failure" group (a) = 10.8 ± 0.6 g.					"
	" " " " "recovery" " (b) = 11.2 ± 0.6 g.					"
0 hours control)	2 + 3		-	7.43 ± 1.02	268.99 ± 9.2	4 +
6 hours	3 + 2		-0.9 ± 0.2	8.65 ± 0.68	291.28 ± 12.6	3 +
4 "	3 + 2		-1.7 ± 0.1	10.46 ± 0.87	200.56 ± 11.3	4 +
8 "(a)	2 + 1		-1.5 ± 0.4	10.86 ± 1.31	94.87 ± 7.5*	3 +
" "(b)	1 + 2		-0.8 ± 0.2	7.73 ± 0.35	198.10 ± 5.0*	2 +
7 days(a)	2 + 2		+0.9 ± 0.7	10.14 ± 0.74*	73.53 ± 5.6*	3 +
" " (b)	1 + 3		+3.3 ± 0.1	8.43 ± 0.30	130.20 ± 5.6*	2 +
1 "		1	+5.5	6.67	125.13	1 +
7 days control)	2 + 3		+5.8 ± 0.2	7.35 ± 0.27 (6.53 ± 0.25)	240.40 ± 13.2 (213.80 ± 12.9)	3 +
1 days control)	5 + 6		+10.5 ± 0.3	6.66 ± 0.22 (6.26 ± 0.20)	141.00 ± 7.7 (132.50 ± 7.5)	5 +

Brackets indicate unadjusted figures, i.e. no allow

*Indicates a significant difference at P = 0.05.

Table 12

3-week-old mice. Strain A, -3°C contro

Time of exposure	Males			
	Mean initial body wt. = 9.1 ± 0.4 g.			
	No. of mice A(V) + A(Y)	Mean body wt. change g.	Mean adrenal wt. mg./100 g.	Mean thymus wt. mg./100 g.
0 hours	3 + 1	-	10.28 ± 0.97	192.78 ± 13.4
24 hours	3 + 3	+0.3 ± 0.0	11.99 ± 1.92	146.00 ± 19.4
48 "	3 + 2	+0.6 ± 0.1	10.78 ± 0.21	117.47 ± 14.7
7 days	3 + 4	+2.1 ± 0.5	11.16 ± 1.06	116.63 ± 18.9

Table 13

3-week-old mice. Strain A. Injected with ACTH for

Total dose	No. of mice A(V) + A(Y)	Mean body wt. change g.	Mean adrenal wt. mg./100 g.	Mean thymus wt. mg./100 g.
	Males			
	Mean initial body wt. = 11.1 ± 0.8 g.			
1.75 mg.	2 + 4	+4.3 ± 0.5	7.16 ± 1.01 (6.37 ± 0.90)	180.91 ± 6.4 (160.87 ± 6.0)
3.5 mg.	5 + 2	+5.4 ± 0.1	7.17 ± 0.23 (6.38 ± 0.20)	126.15 ± 12.2* (112.24 ± 11.1)
7.0 mg.	4 + 2	+4.8 ± 0.5	8.06 ± 0.47 (7.17 ± 0.42)	80.48 ± 4.6* (71.58 ± 4.1)
10.5 mg.	2 + 2	+4.5 ± 0.3	9.20 ± 0.33* (8.18 ± 0.29)	79.40 ± 8.5* (70.62 ± 7.6)
- (control)	2 + 3	+5.8 ± 0.2	7.35 ± 0.27 (6.53 ± 0.25)	240.40 ± 13.2 (213.80 ± 12.9)

Brackets indicate unadjusted figures, i.e.

*Indicates a significant difference at P = 0

Table 14

Comparison of relative adrenal weight in

		Males			
		A(V)		A(Y)	
Age (weeks)	Time of exposure	No. of mice	Mean adrenal wt. mg./100 g.	No. of mice	Mean adre wt. mg./10
12	21 days	2	5.62	3	5.38
"	21 " (control)	3	3.97	4	4.11
5	21 days	2	7.05	3	8.23
"	21 " (control)	2	5.90	3	5.99
"	0 hours (-3°C control)	2	7.19	2	7.81
3	48 hours (a)	2	11.20	1	10.02
	" " (b)	1	7.27	2	7.98
"	7 days (a)	2	9.83	2	10.45
	" " (b)	1	8.57	3	8.40
"	7 " (control)	2	7.01	3	7.57
"	0 hours (-3°C control)	3	10.63	1	9.67

Appendix B.

Figures 1 - 52.

The following abbreviations are used in the captions of the Figures:

S.B.	-	Stained with Sudan black
S.4.	-	Stained with Sudan 4 and Erlich's haematoxylin
H. & E.	-	Stained with Mayer's haemalum and eosin
M.	-	Stained with Masson's trichrome
Schultz	-	Stained with the Schultz stain for cholesterol
w.	-	week-old
hr.	-	hours of exposure
d.	-	days of exposure

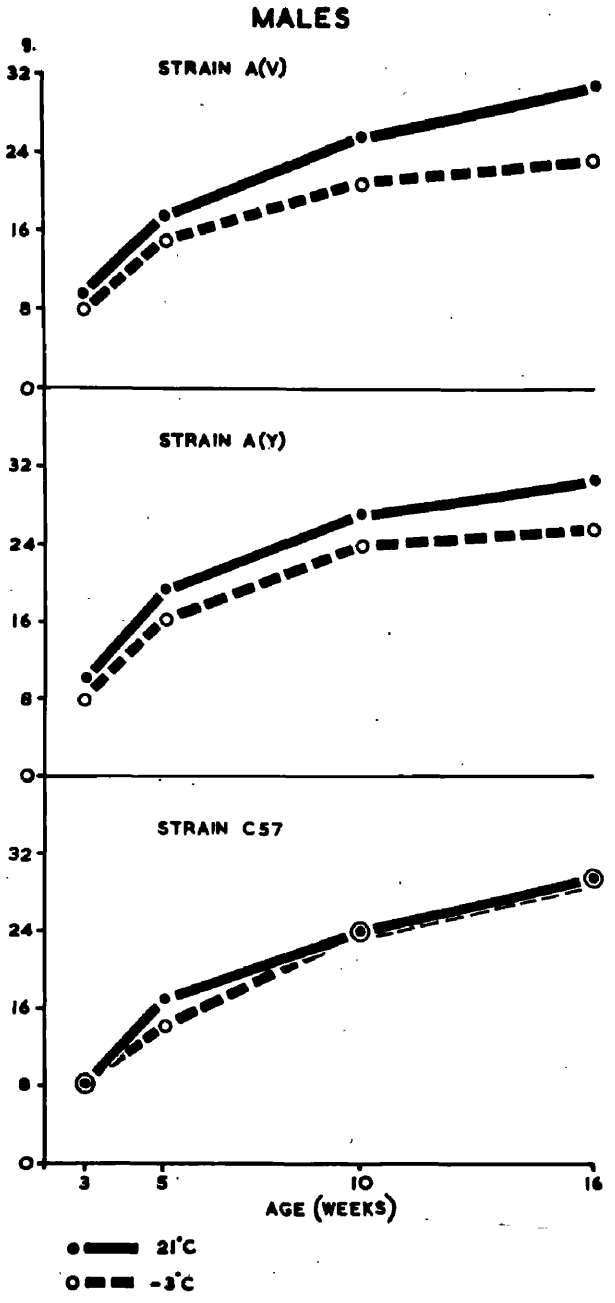

Fig.1. Graph illustrating the growth of 1st generation male mice.(p.11)

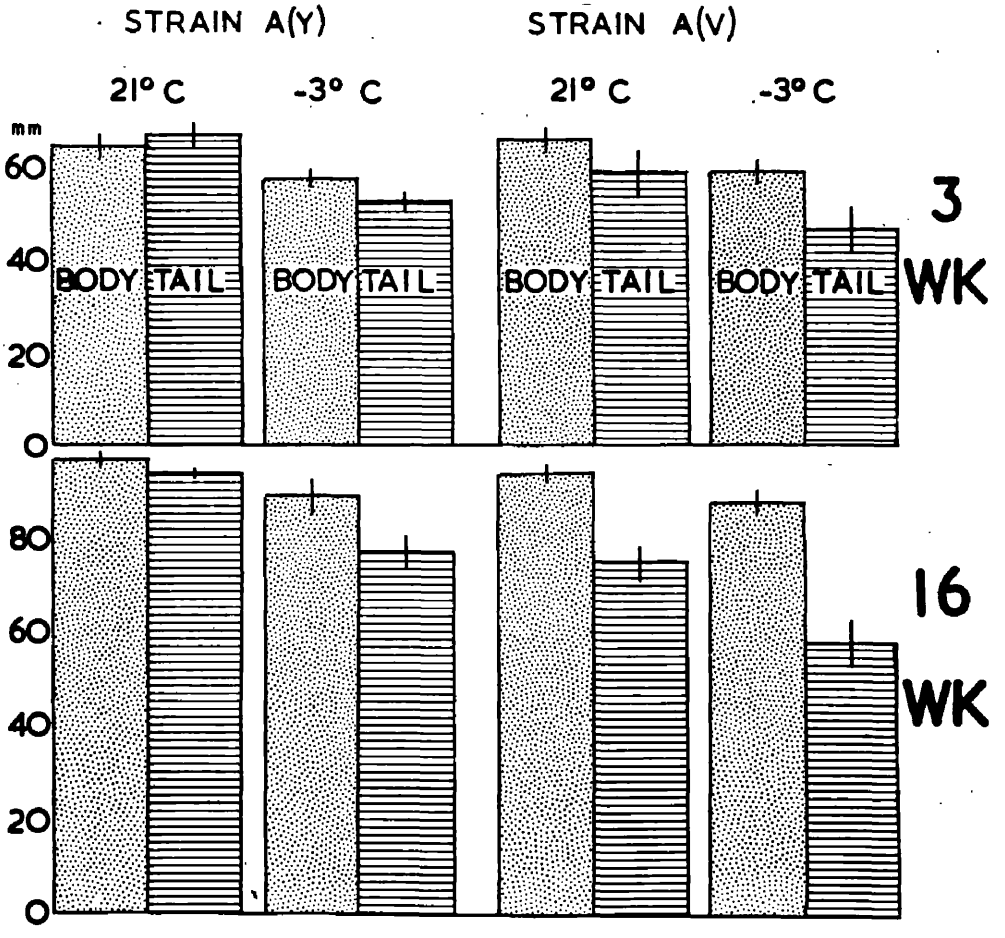

Fig.2. Histogram illustrating the body and tail lengths of A strain mice. (p.15)

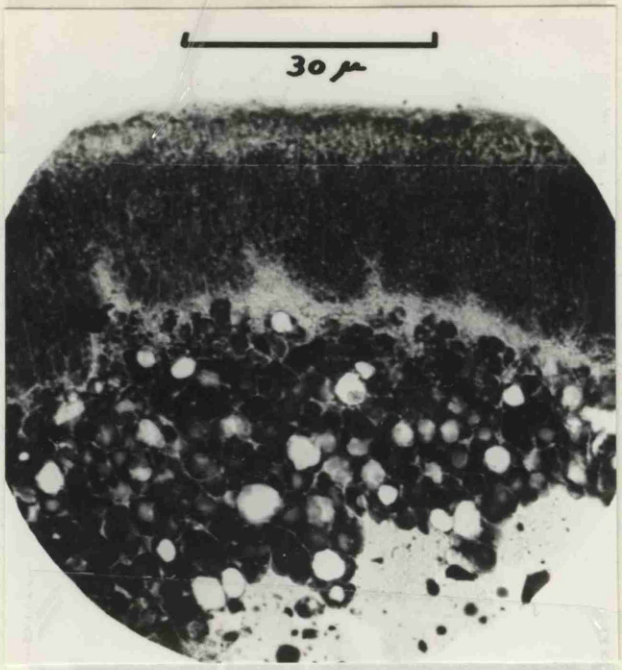

Fig. 3. Adrenal. Unmated female 21°C, A strain. S.B.

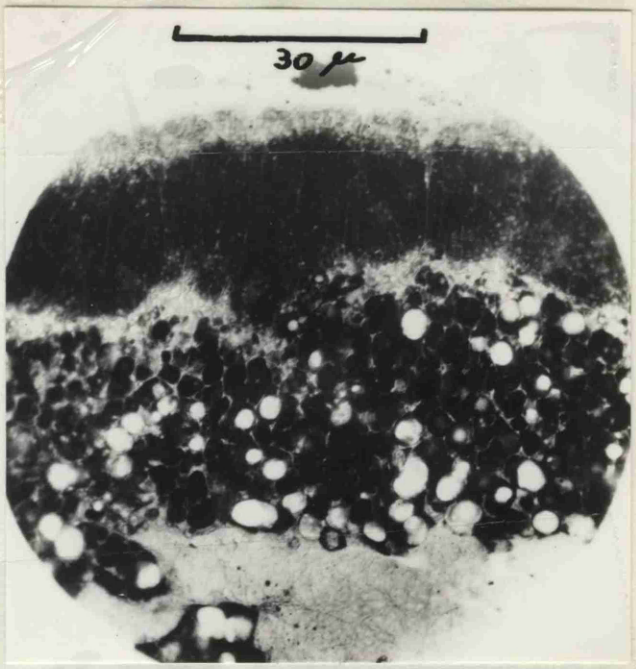

Fig. 4. Adrenal. Unmated female -3°C, A strain. S.B.

No depletion from zona fasciculata at either temperature, zona glomerulosa lightly stained. (p.22)

Fig. 5. Adrenal. Breeding female 21°C, A strain. S.B.

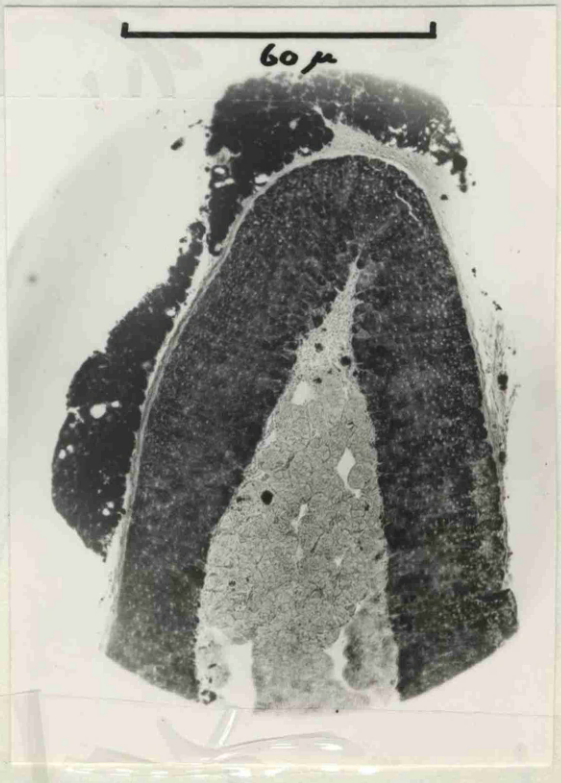

Fig. 6. Adrenal. Breeding female -3°C, A strain. S.B.

No depletion of lipid from cortex at either temperature.(p.22)

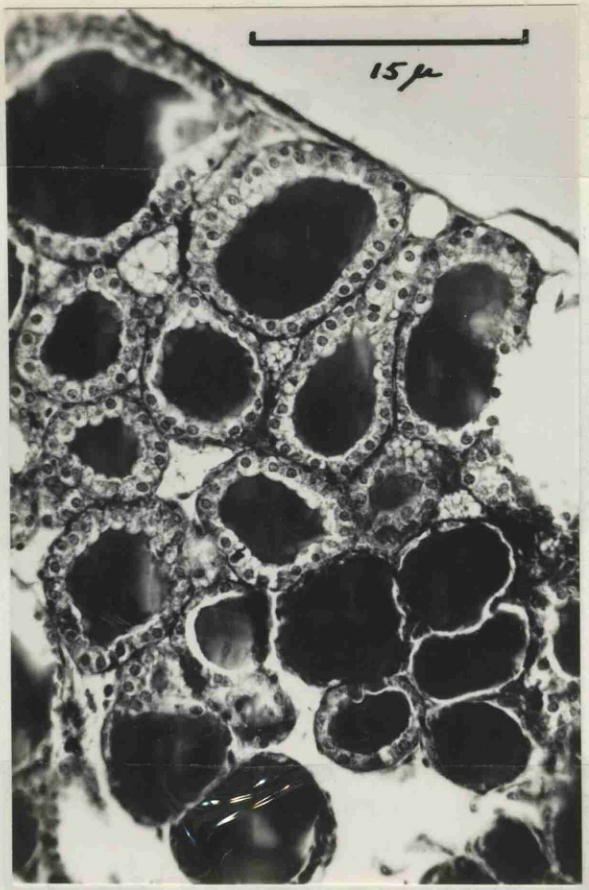

Fig. 7. Thyroid. Unmated female 21°C, A strain. M.

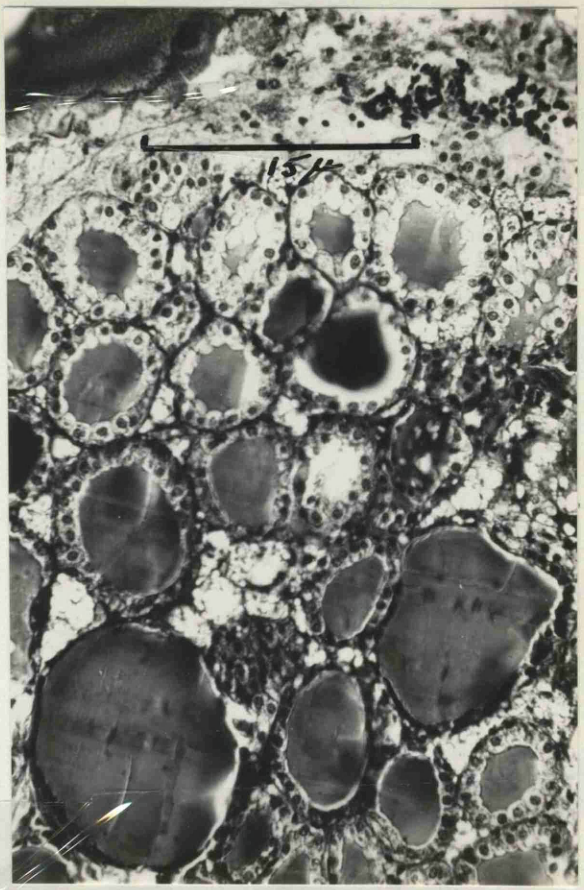

Fig. 8. Thyroid. Unmated female -3°C, A strain. M.

The normal mixture of active and inactive follicles at both temperatures. (p.22)

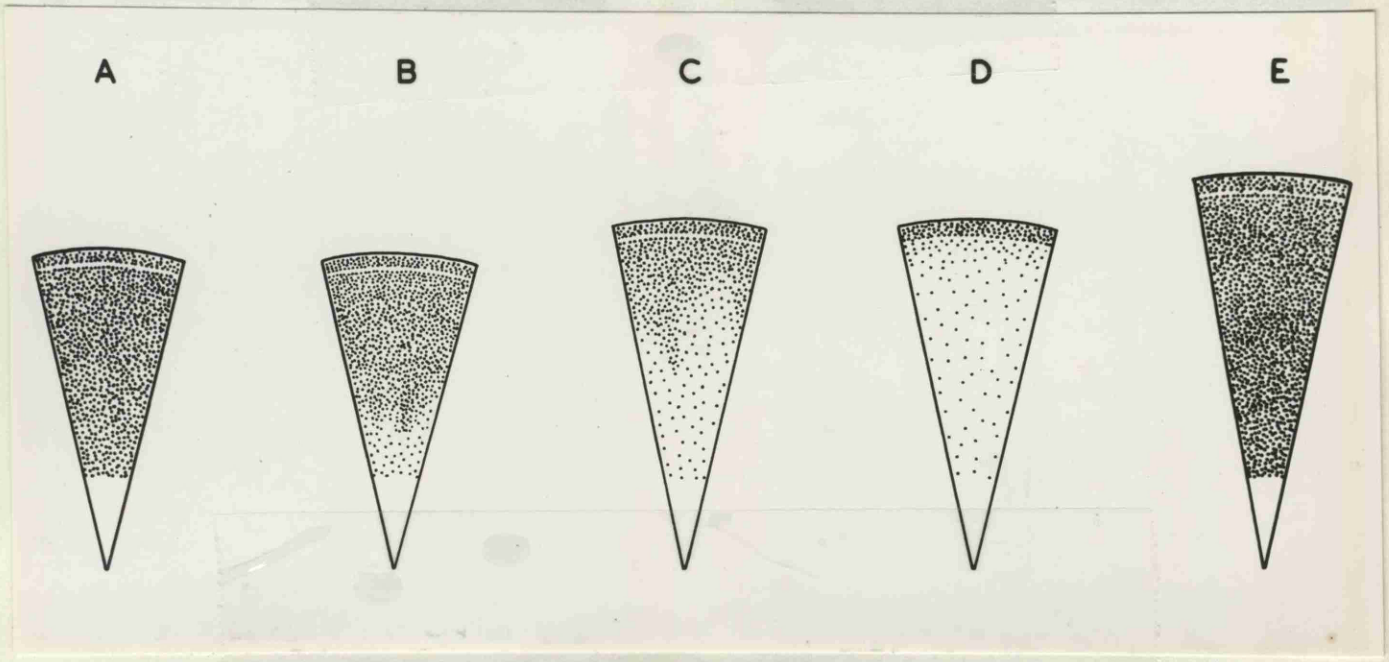

Fig.9. Diagram illustrating changes in the adrenal gland of a mammal during exposure to cold. (See text p.34-35)

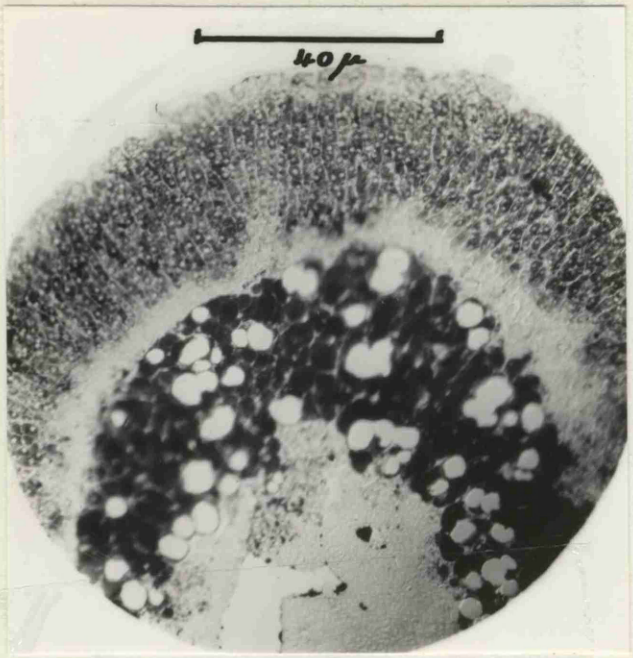

Fig. 10. Adrenal. 12 w. female 24 hr. S.B. (Partial depletion)

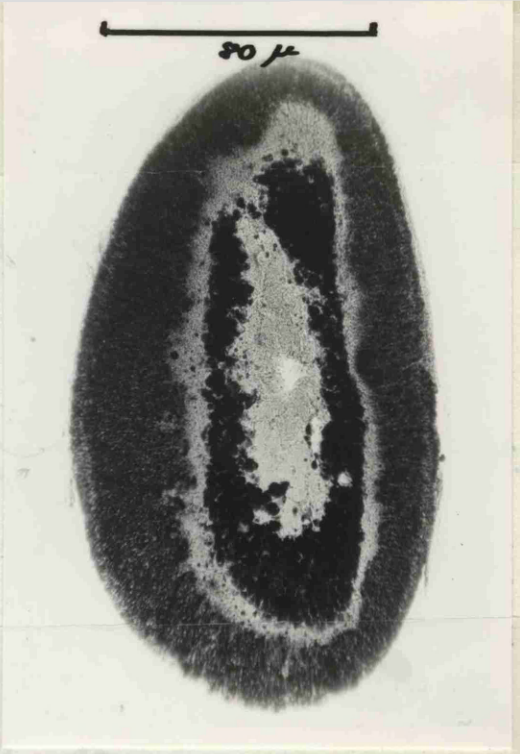

Fig. 11. Adrenal. 12 w. female 7 d. S.B. (Recovery)

Typical "alarm" reaction immediately after exposure, followed by the "recovery" stage. (p.35)

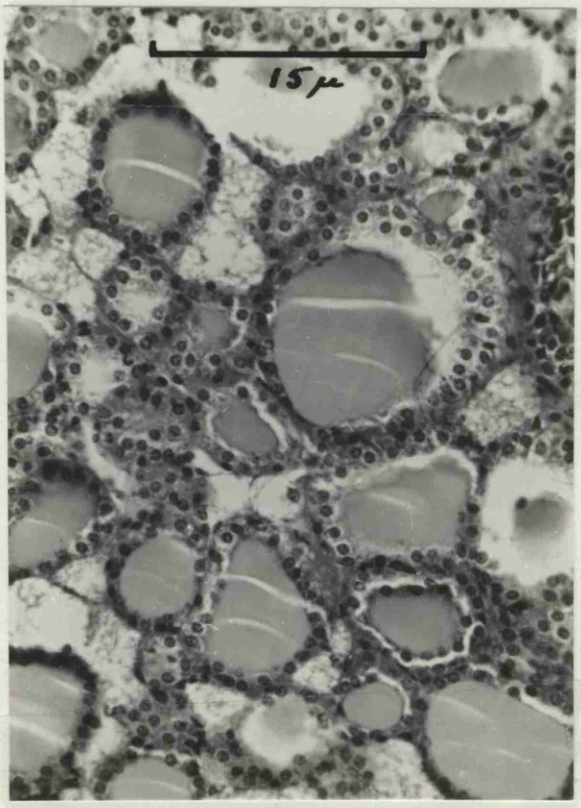

Fig. 12. Thyroid. 12 w. female 0 hr. H. & E. (Active and inactive follicles)

Fig. 13. Thyroid. 12 w. female 6 hr. H. & E. (Active)

Rapid increase in thyroid activity after exposure.(p.35)

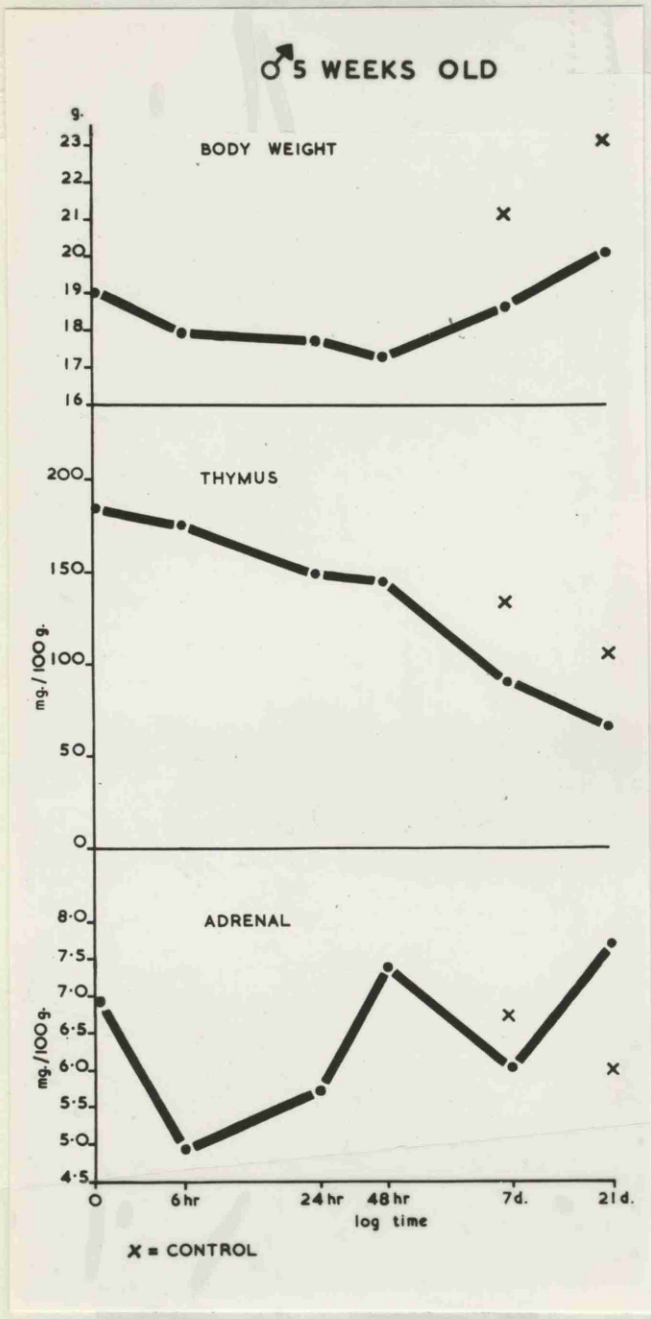

Fig. 14.

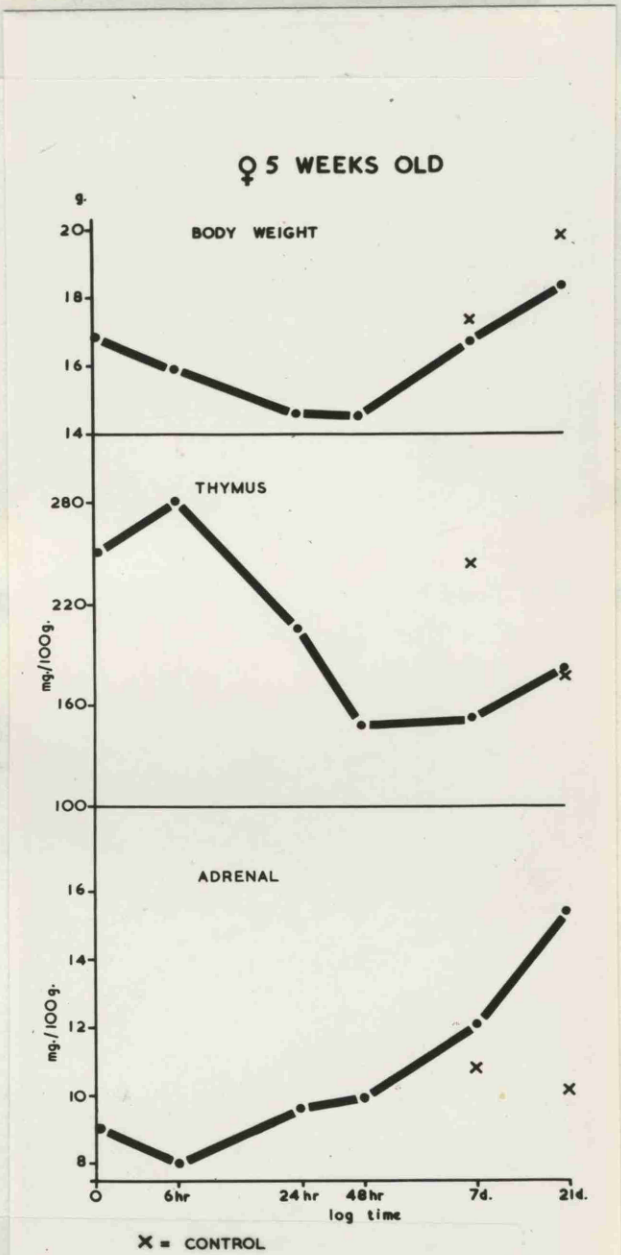

Fig. 15.

Graphs illustrating growth, thymus and adrenal weights of 5-week-old mice.

Mean body weight at 0 hours includes both experimental and control mice. (p.36,40)

Fig. 16. Adrenal. 5 w. male A strain 6 hr. S.B. (Partial depletion)

Fig. 17. Adrenal. 5 w. male A strain 7 d. S.B. (Recovery)

"Alarm" reaction followed by "recovery" stage. (p.40)

Fig. 18. Adrenal. 5 w. male A strain 6 hr. S.B.

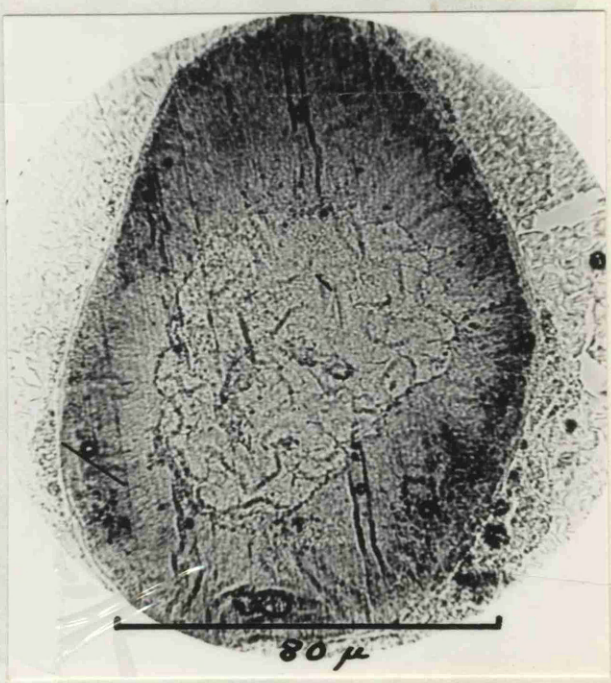

Fig. 19. Adrenal. 5 w. male A strain 6 hr. Schultz.

The same area is stained in each case; this indicates that the sudanophilic substance in the zona fasciculata is cholesterol. (p.40)

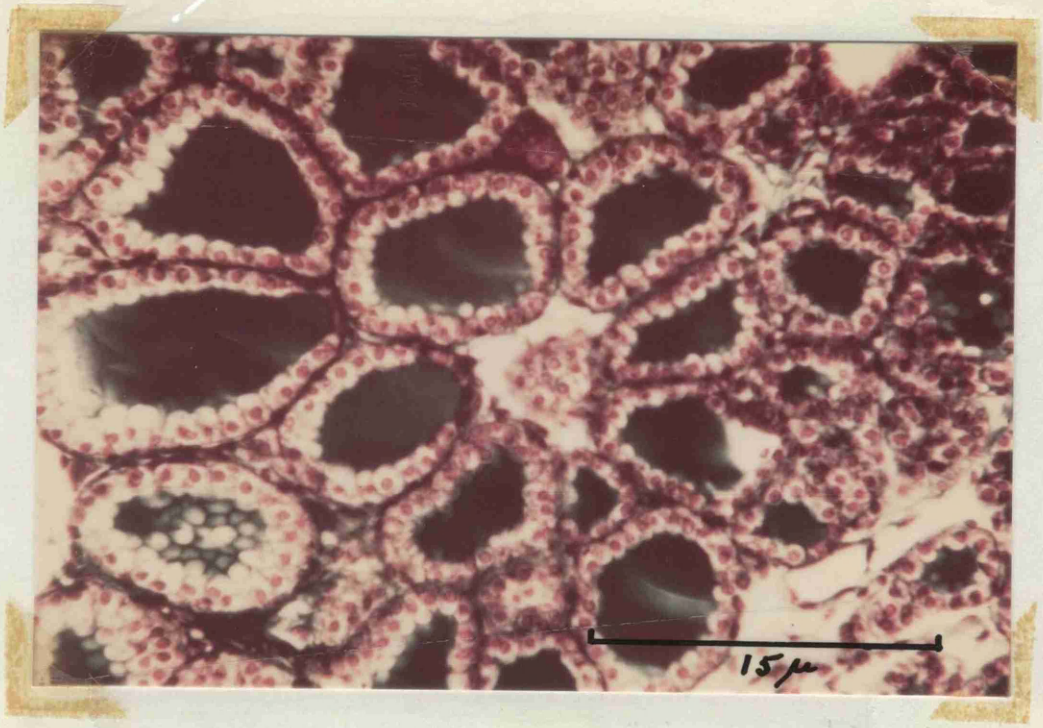

Fig. 20. Thyroid. 5 w. male A strain 48 hr. M. (Active)

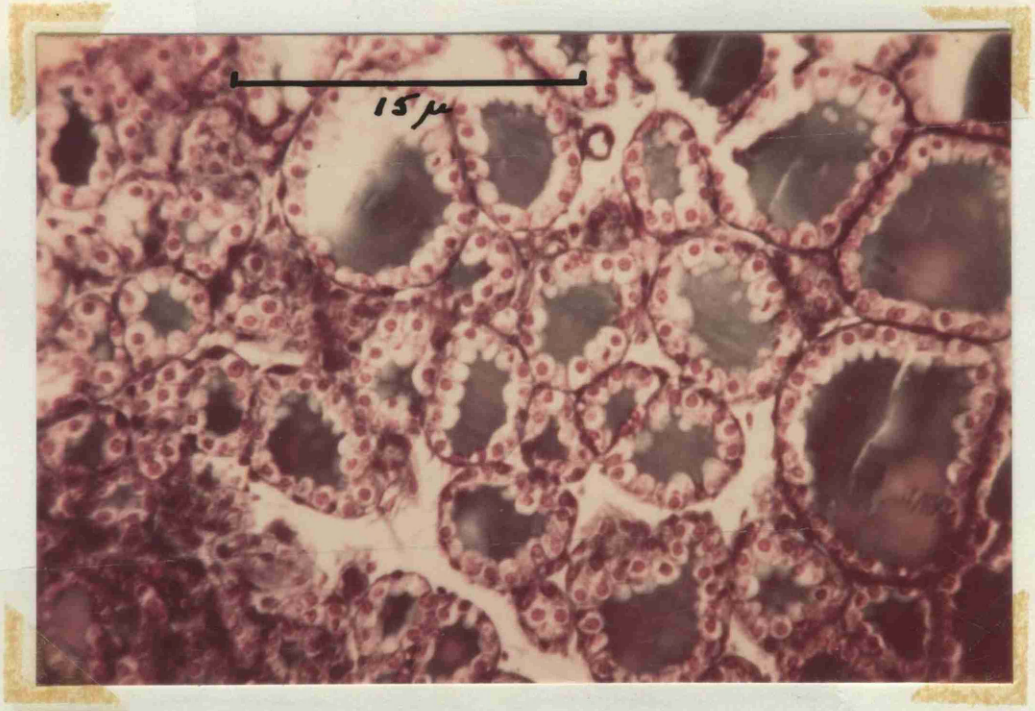

Fig. 21. Thyroid. 5 w. male A strain 7 d. M. (Still very active)

The immediate increase in thyroid activity is still present after 7 days' exposure. (p.41)

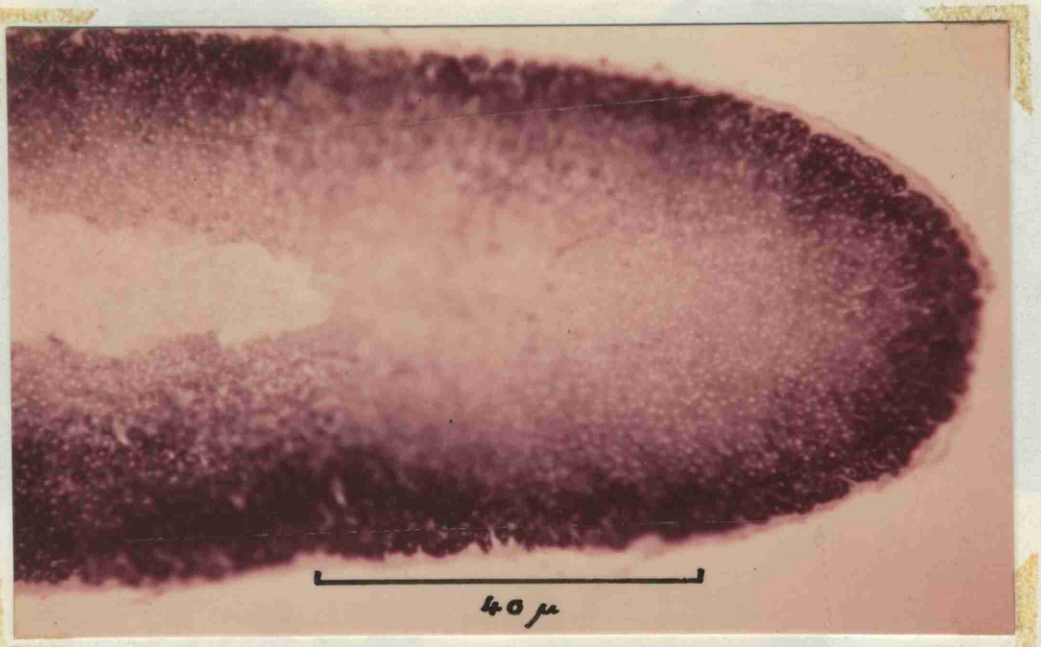

Fig. 22. Adrenal. 5 w. male A strain 7 d. S.B. (Severely depleted)

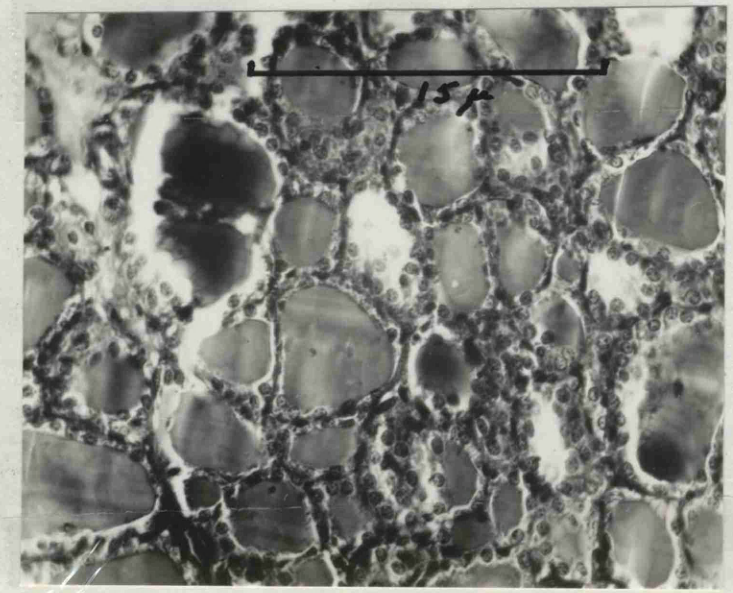

Fig. 23. Thyroid. 5 w. male A strain 7 d. M. (Inactive)

The single 5-week-old male which failed to regain its initial body weight, showed a prolonged and severe "alarm" reaction of the adrenal gland and a reduction in thyroid activity. (p.41)

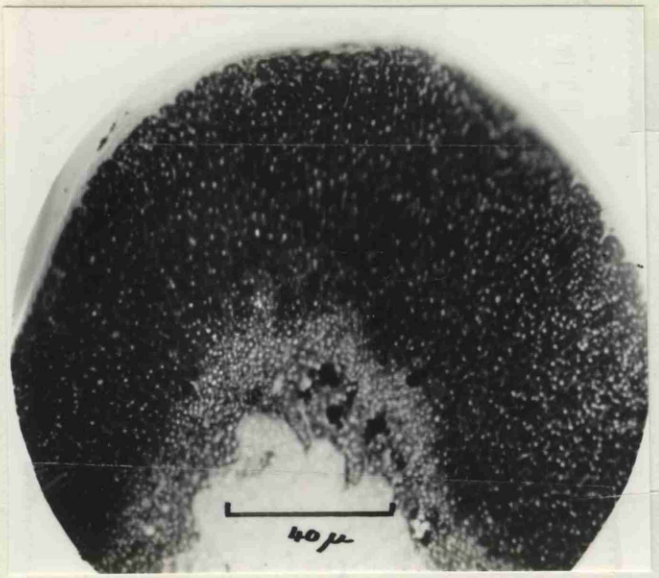

Fig. 24. Adrenal. 5 w. -3°C control female A strain 48 hr. S.B.

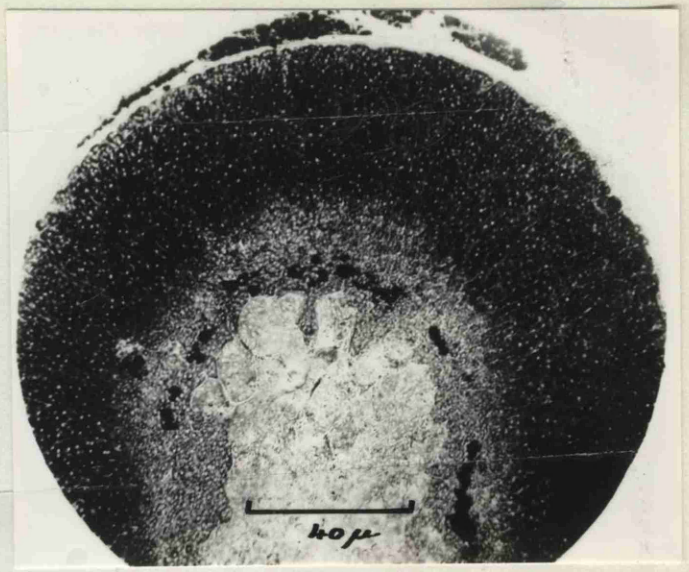

Fig. 25. Adrenal. 5 w. -3°C control female A strain 7 d. S.B.

No depletion of lipid from cortex. (p.42)

Fig. 26. Adrenal. 5 w. female GFF strain 24 hr. S.B. (Partial depletion)

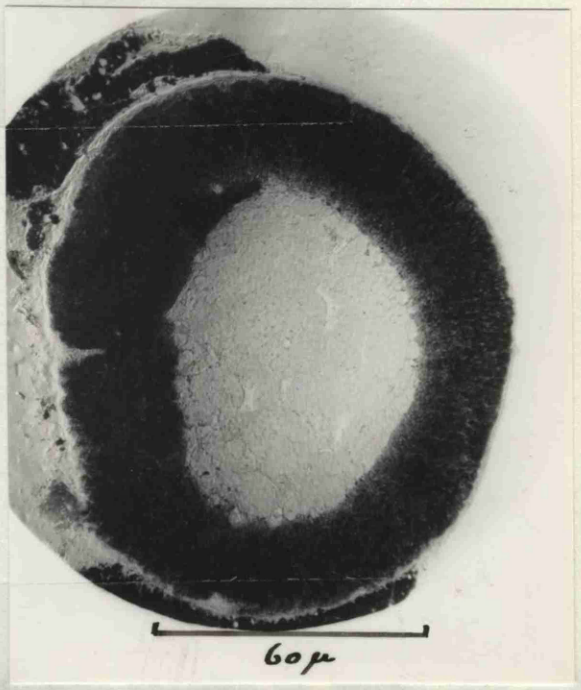

Fig. 27. Adrenal. 5 w. male GFF strain 7 d. S.B. (Recovery)

Typical "alarm" reaction immediately after exposure, followed by a "recovery" stage in a mouse that survived for 7 days. (p.45)

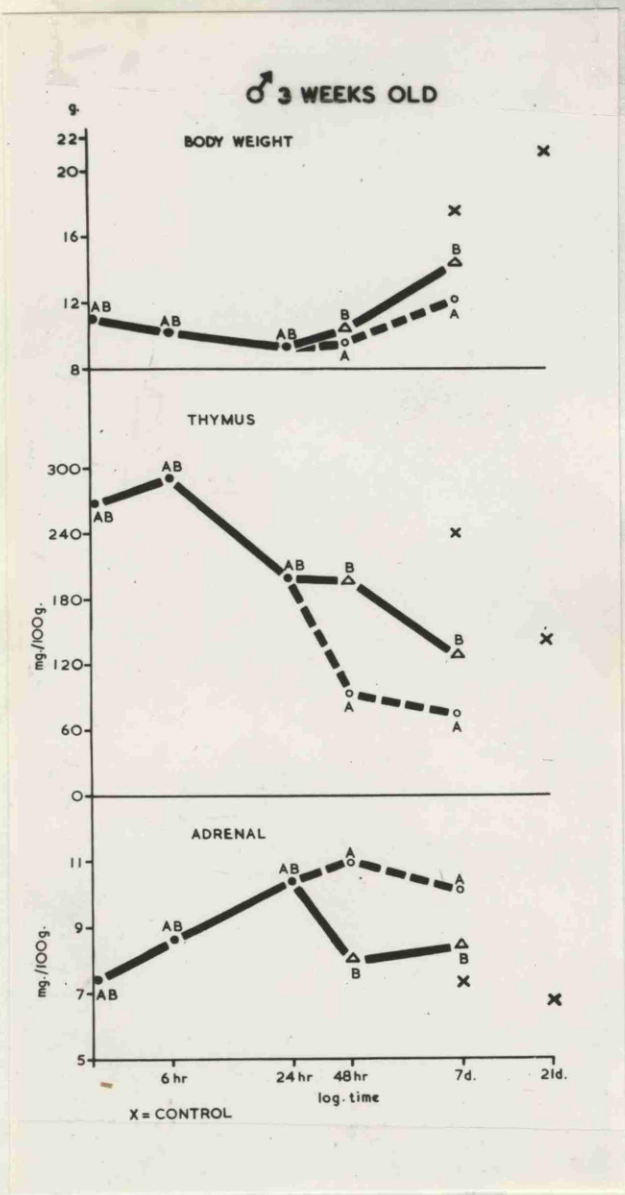

Fig. 28.

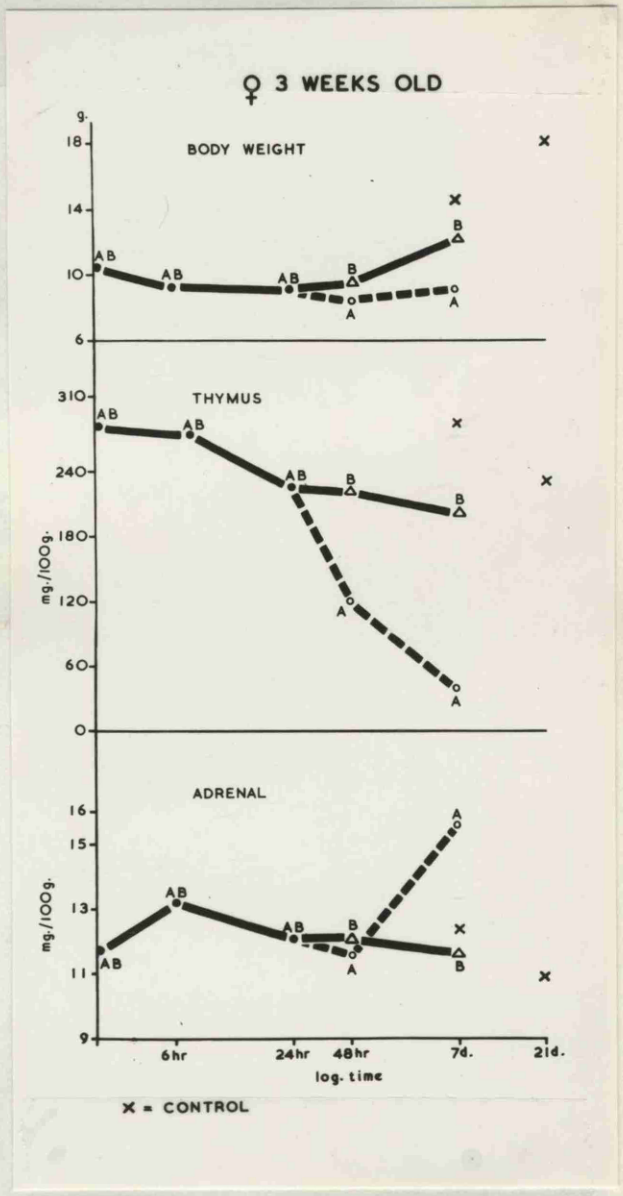

Fig. 29.

Graphs illustrating growth, thymus and adrenal weights of 3-week-old mice.

Mean body weight at 0 hours includes both experimental and control mice. (p.46)

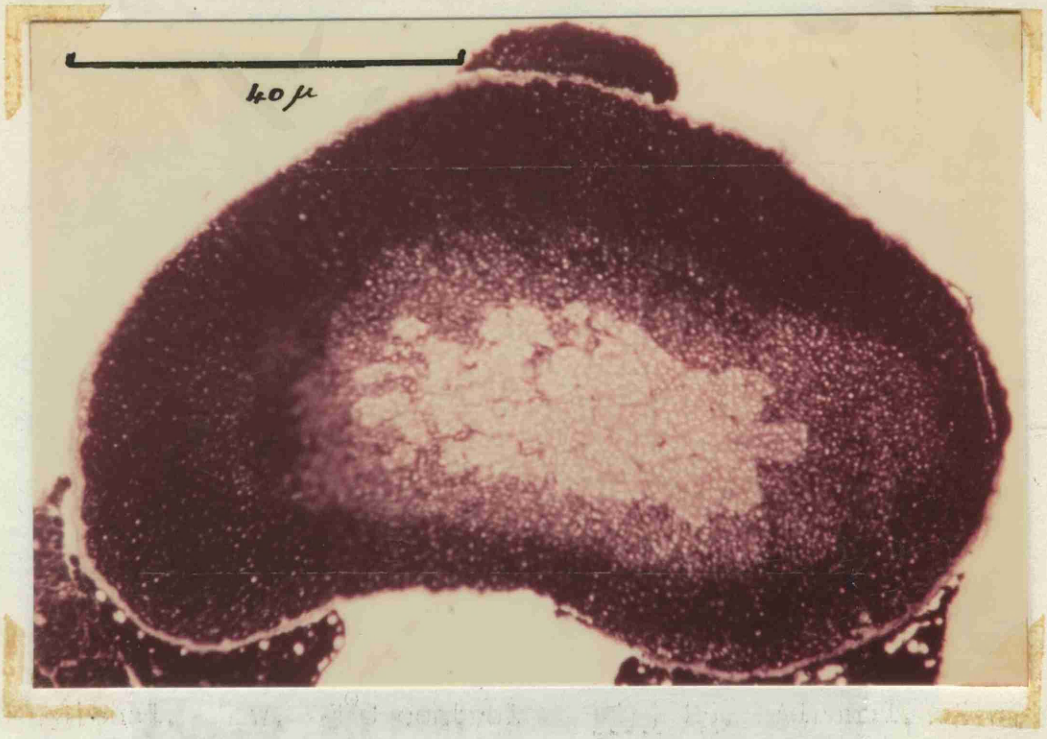

Fig. 30. Adrenal. 3 w. female 0 hr. S.B. (Normal gland)

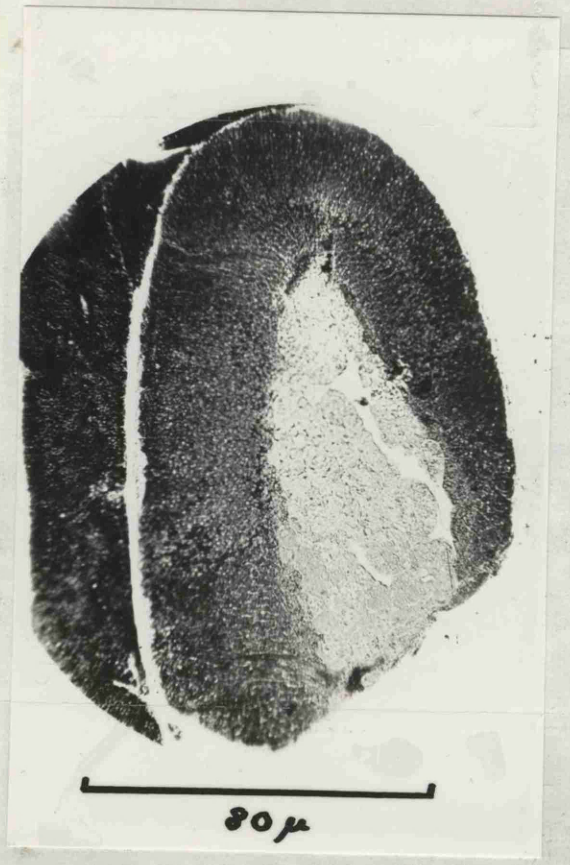

Fig. 31. Adrenal. 3 w. female 6 hr. S.B. (Partial depletion)

Fig. 32. Adrenal. 3 w. female 7 d. S.B. (Recovery)

The normal sequence of changes in the adrenal gland of a mouse of the "survival" group (b). (p.48)

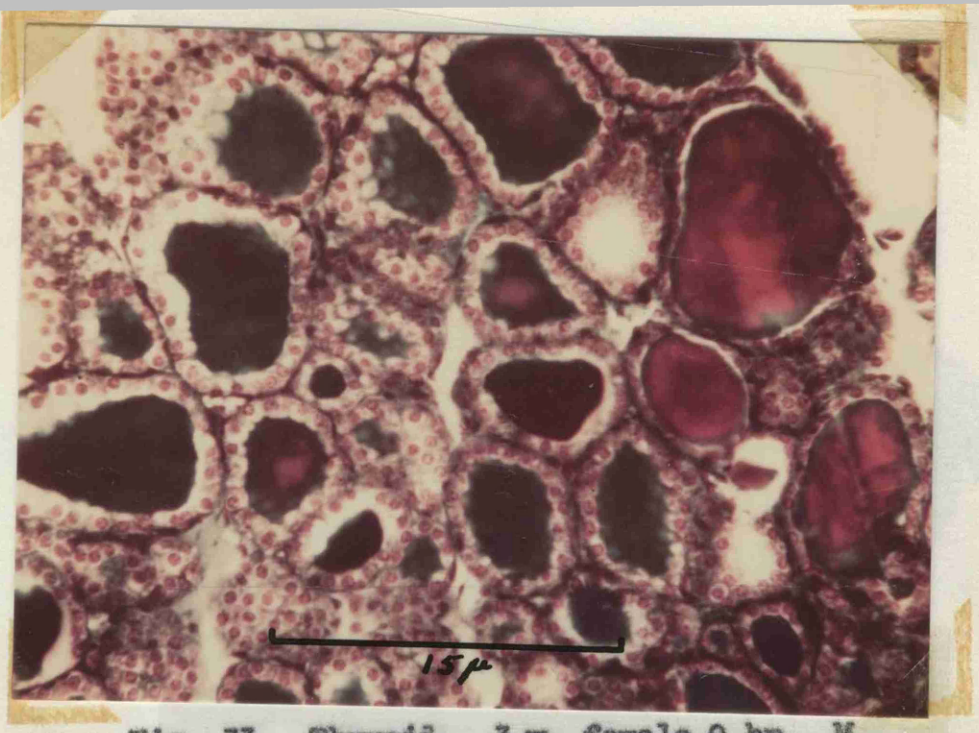

Fig. 33. Thyroid. 3 w. female 0 hr. M. (Active and inactive follicles)

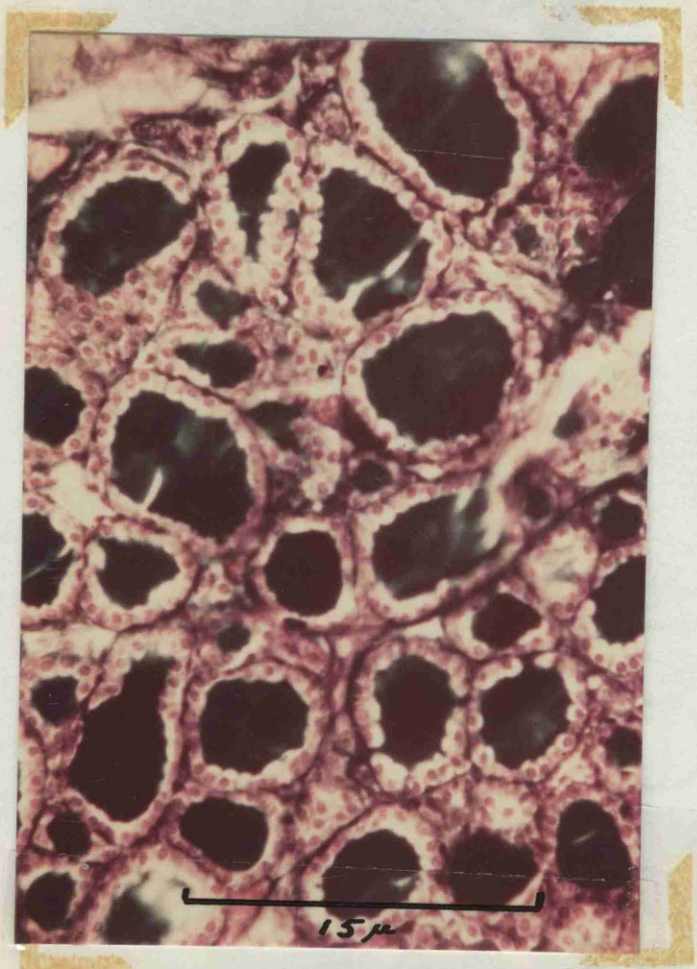

Fig. 34. Thyroid. 3 w. female 7 d. M. (Active)

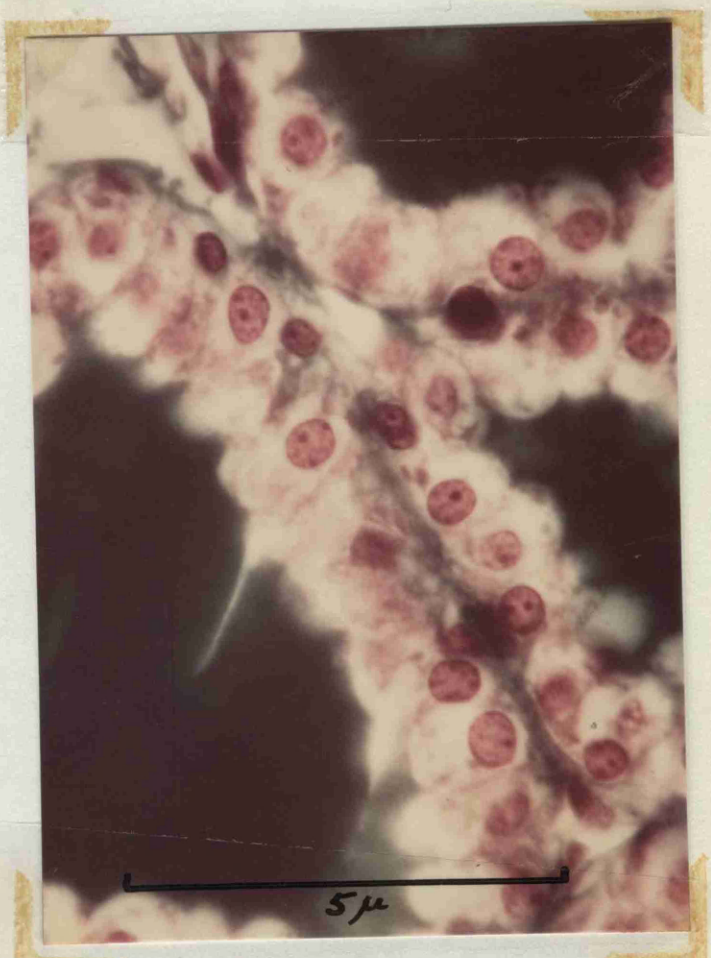

Fig. 35. Thyroid. 3 w. female 7 d. M. (Active)

Marked increase in thyroid activity in mouse of the "survival" group (b). (p.48)

Fig. 36. Adrenal. 3 w. female 7 d. S.B. (Severely depleted)

Prolonged and severe "alarm" reaction in mouse of the "failure" group (a). (p.48)

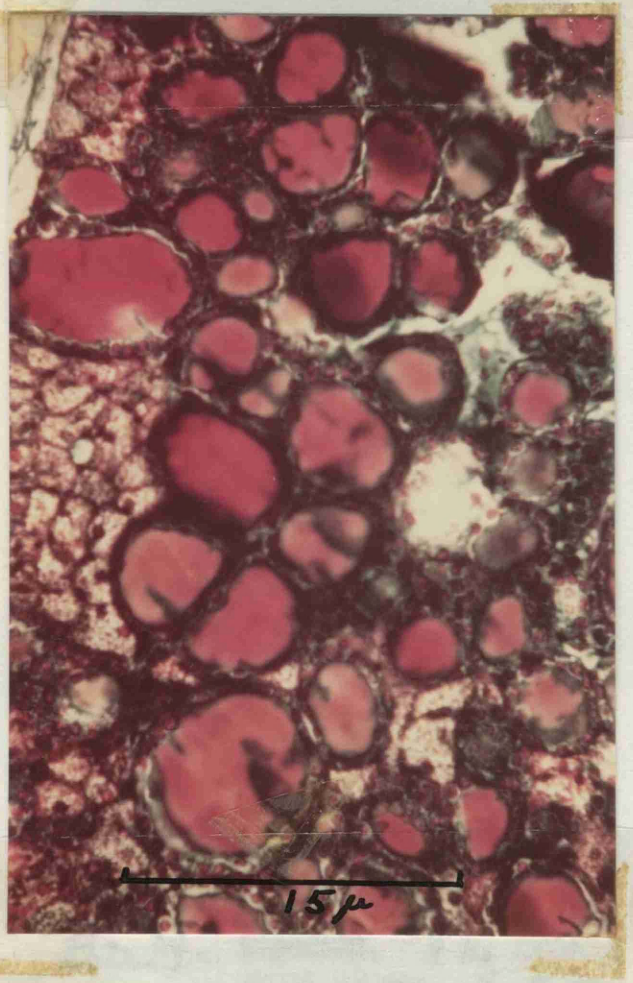

Fig. 37. Thyroid. 3 w. female 7 d. M. (Very inactive)

Fig. 38. Thyroid. 3 w. female 7 d. M. (Very inactive)

Very inactive thyroid gland in mouse of "failure" group (a). (p.48)

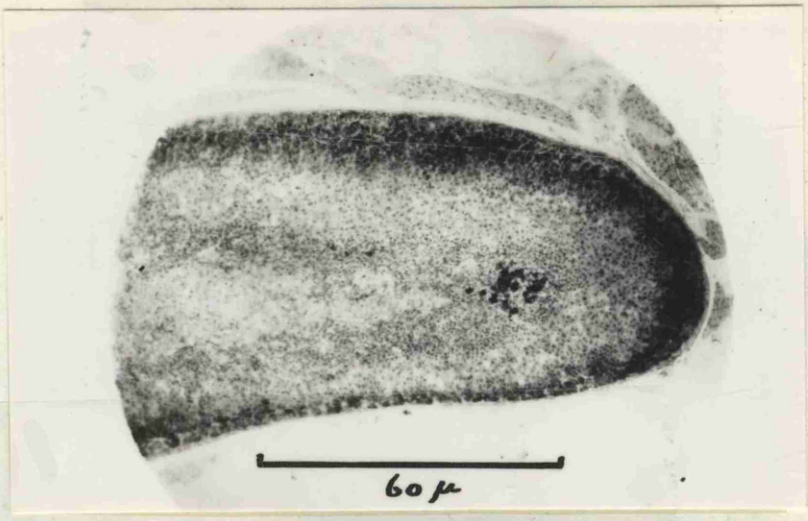

Fig. 39. Adrenal. 3 w. female died 48 hr. S.4. (Severely depleted)

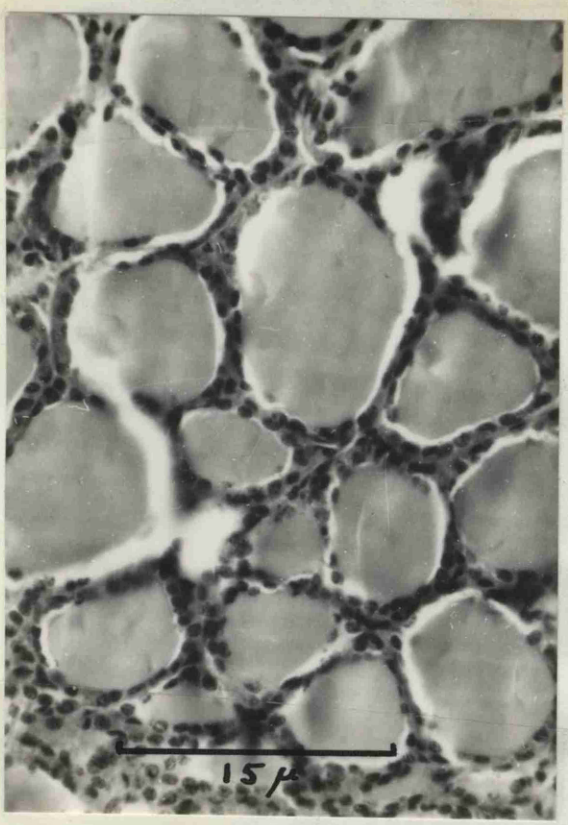

Fig. 40. Thyroid. 3 w. female died 48 hr. H. & E. (Very inactive)

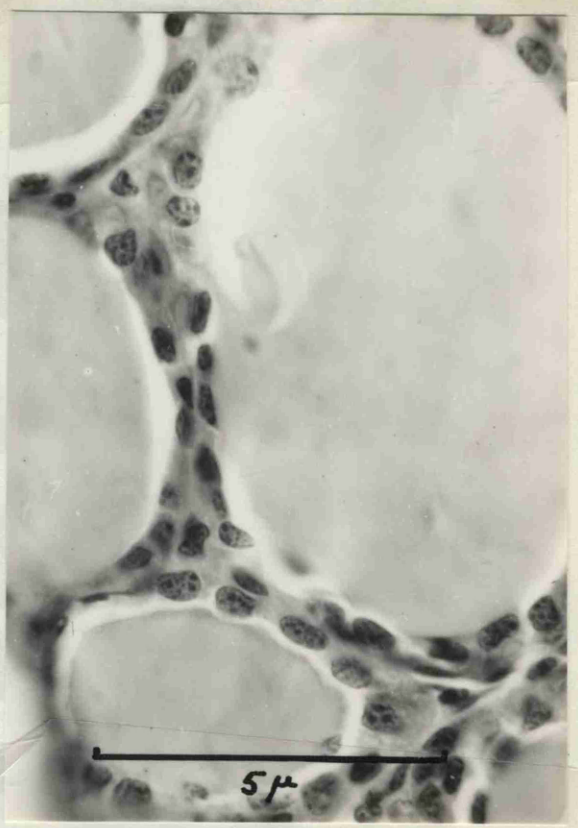

Fig. 41. Thyroid. 3 w. female died 48 hr. H. & E. (Very inactive)

Note marked similarity of adrenal and thyroid glands to those of the "failure" group (a) mice. (p.49)

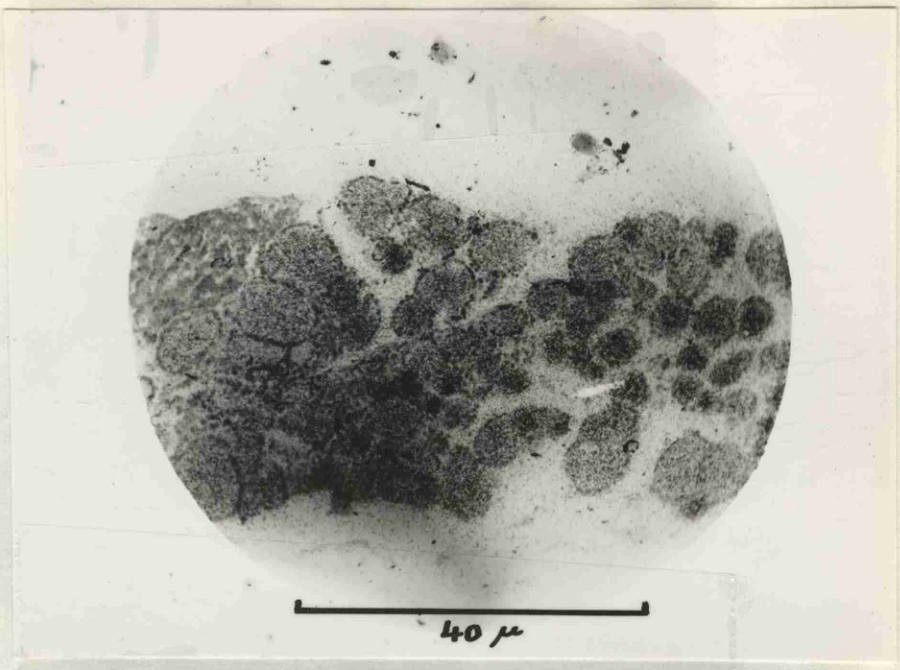

Fig.43. Thyroid. 3w. male 7d. Autoradiograph.

Inactive gland and low concentration of I^{131}.(p.53)

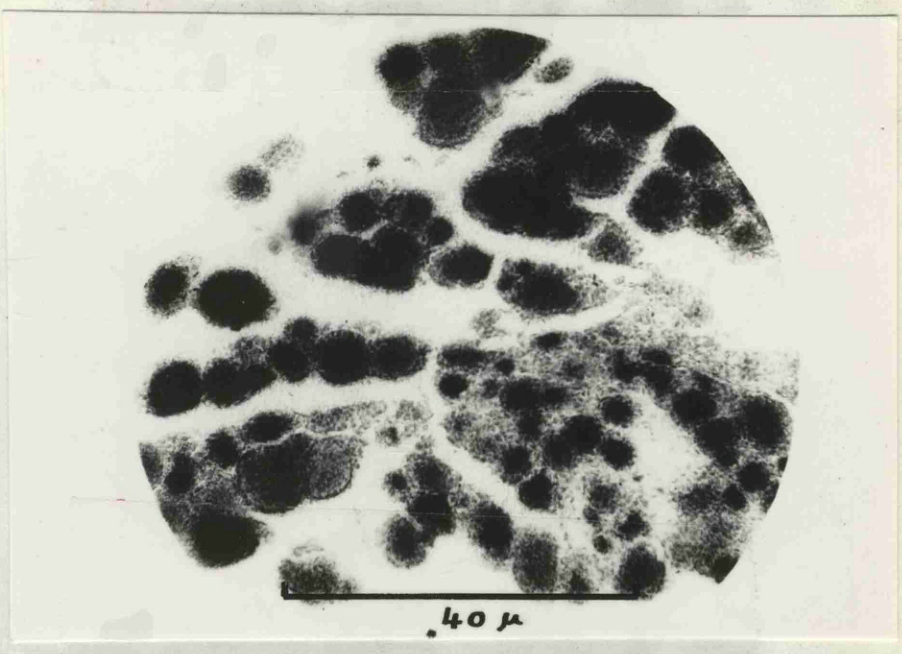

Fig.42. Thyroid. 3w. male 7d. Autoradiograph.

Active gland and high concentration of I^{131}.(p.53)

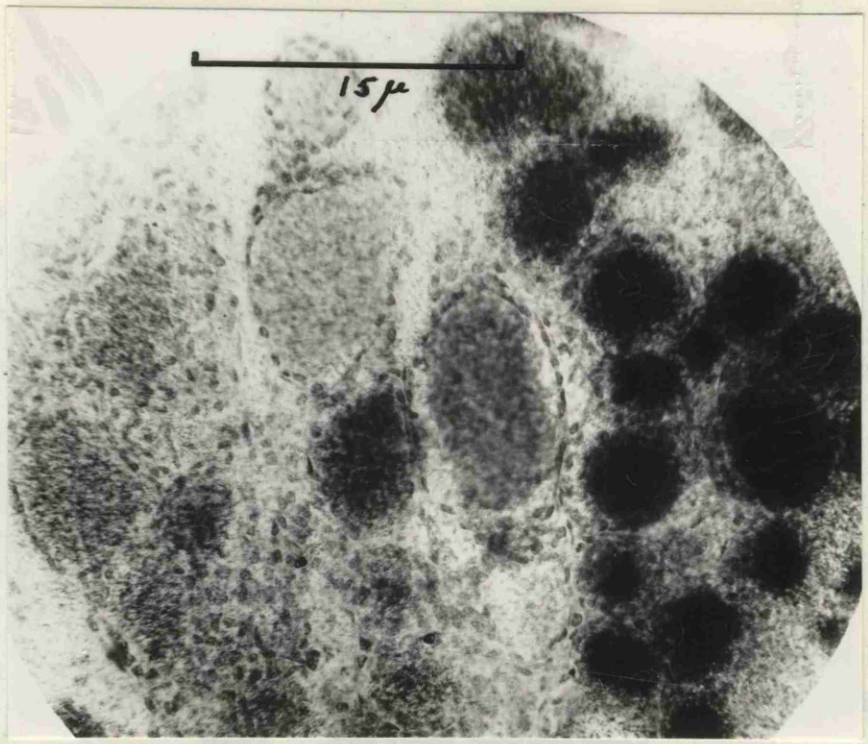

Fig. 44. Thyroid. 3 w. male 21°C control 7 d. Autoradiograph.

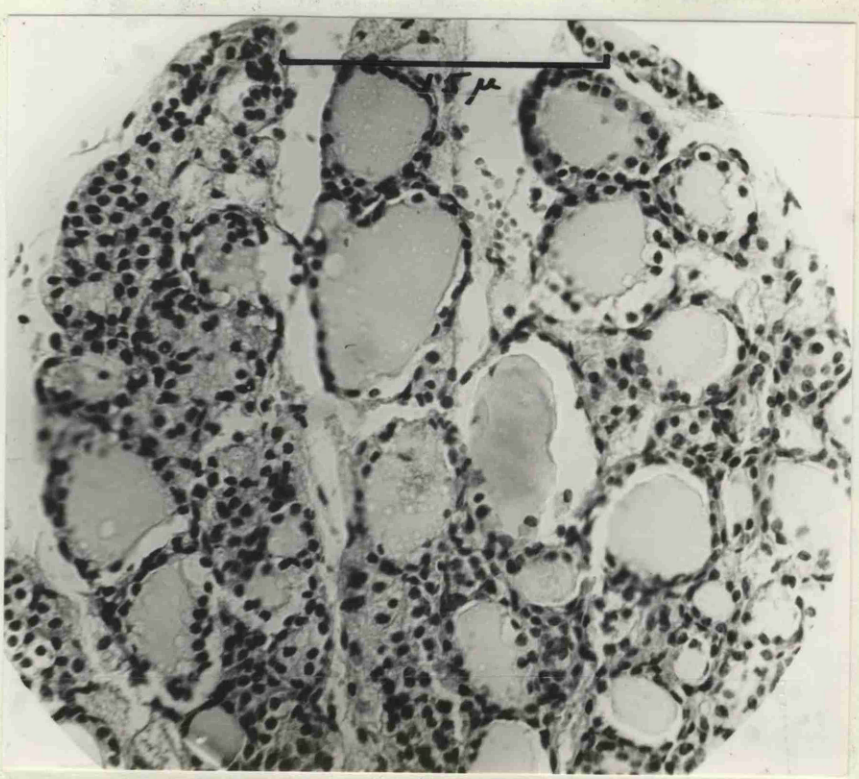

Fig. 45. Thyroid. 3 w. male 21°C control 7 d. H. & E.

Active follicles corresponding with high concentration I^{131}, resting follicles corresponding with low concentration I^{131}. (p.53)

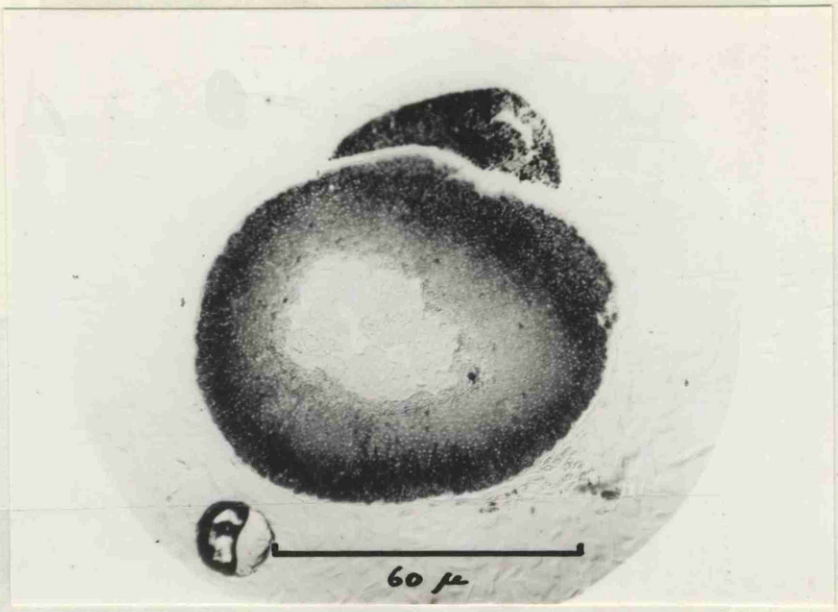

Fig. 46. Adrenal. 3 w. male -3°C control 48 hr. S.B. (Partial depletion)

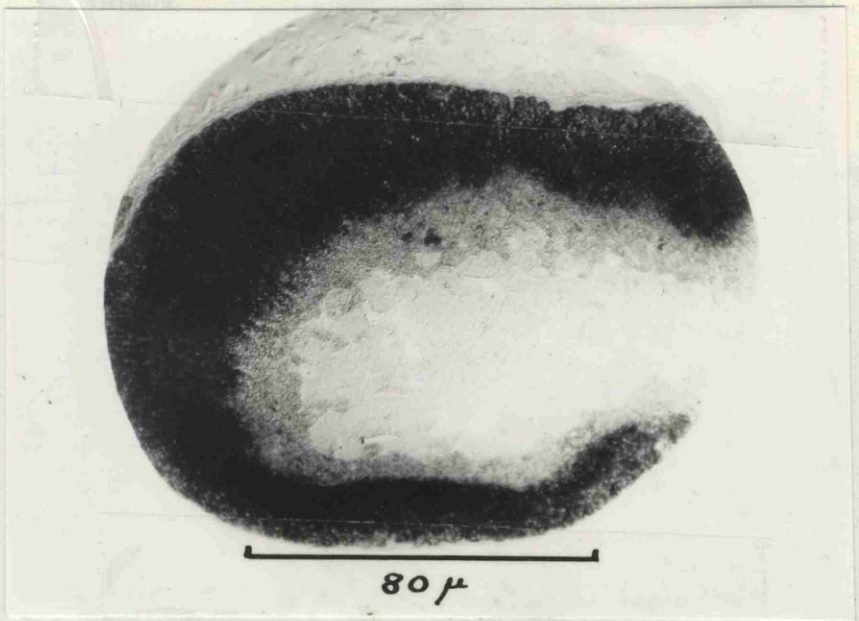

Fig. 47. Adrenal. 3 w. female -3°C control 7 d. S.B. (Recovery)

The typical "recovery" stage after an initial period of partial depletion. (p.50)

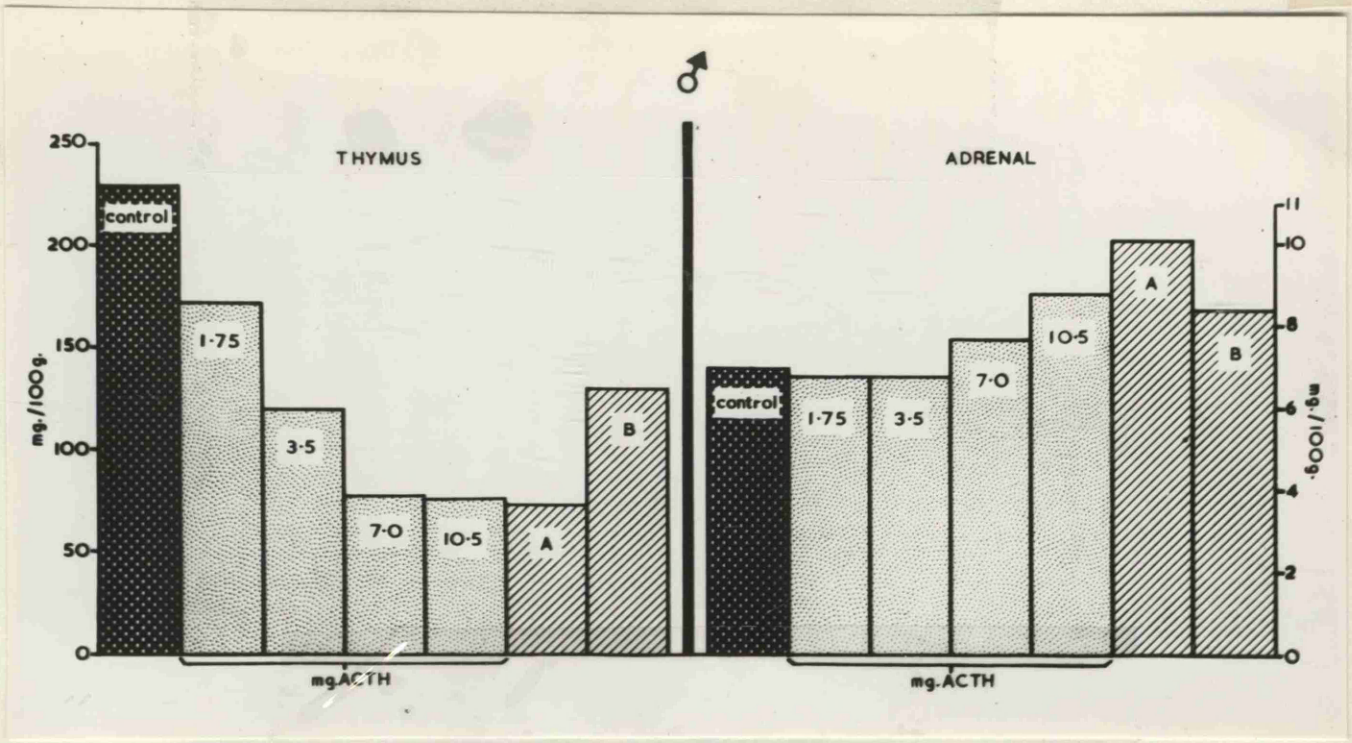

Fig. 48.

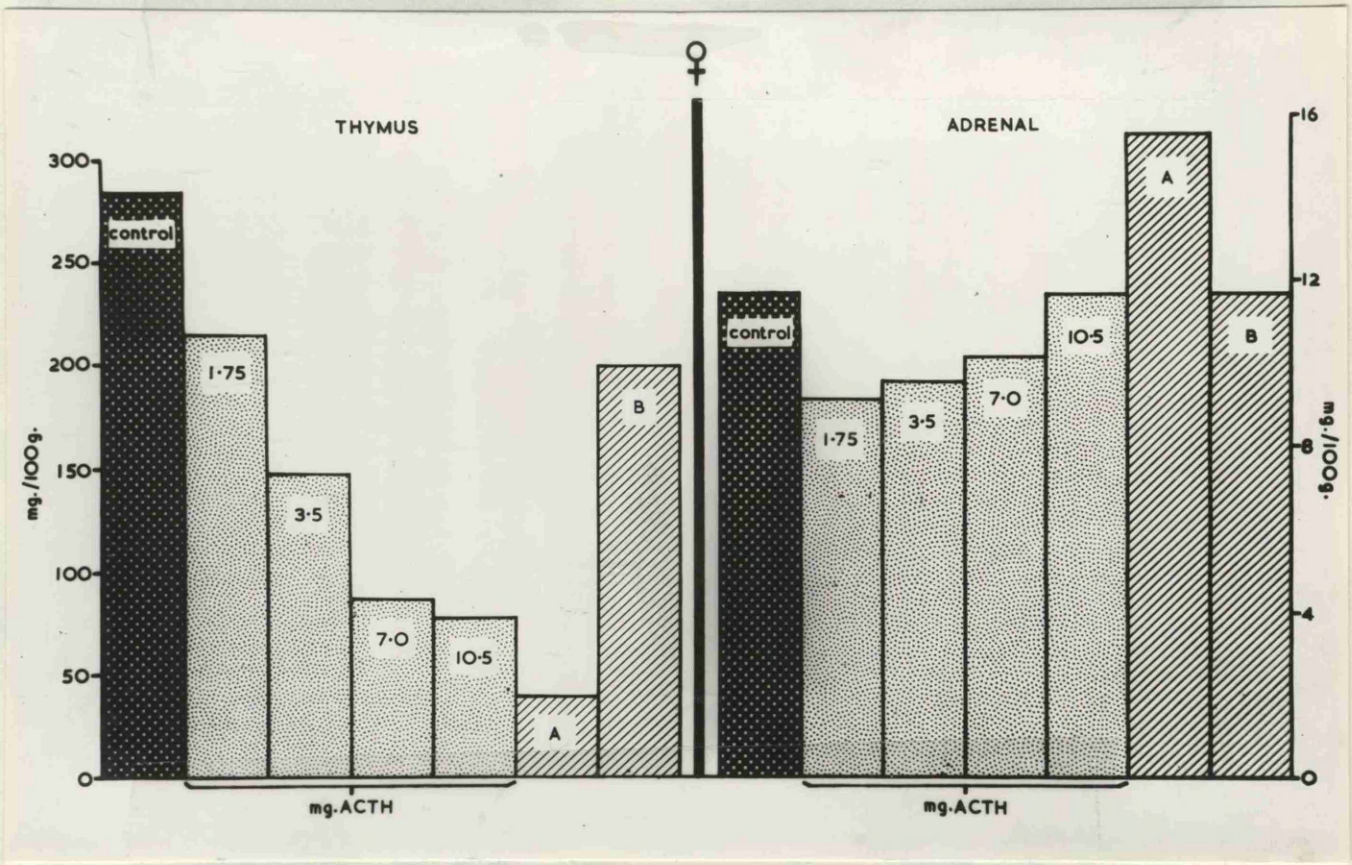

Fig. 49.

Histograms illustrating the effects of exposure to cold or injections with ACTH at 21°C. (p.59)

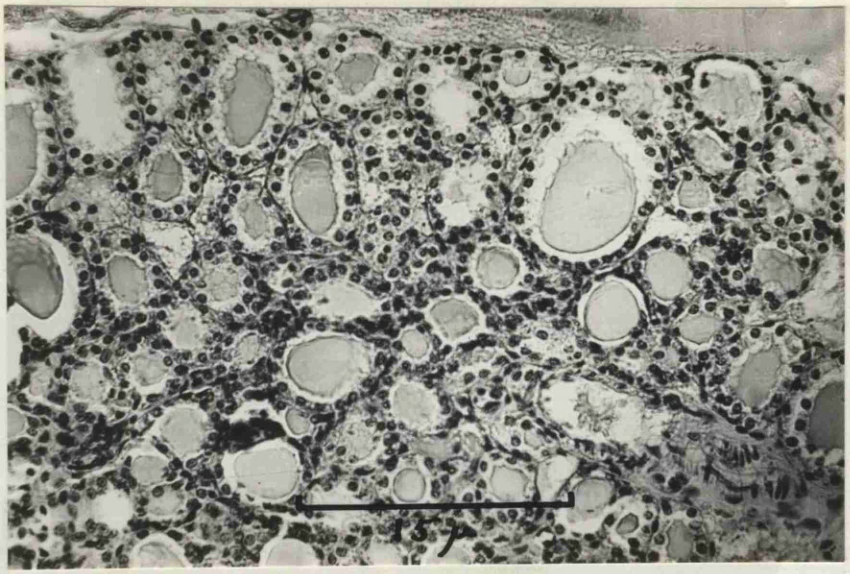

Fig. 50. Thyroid. 3 w. female 21°C control. H. & E.

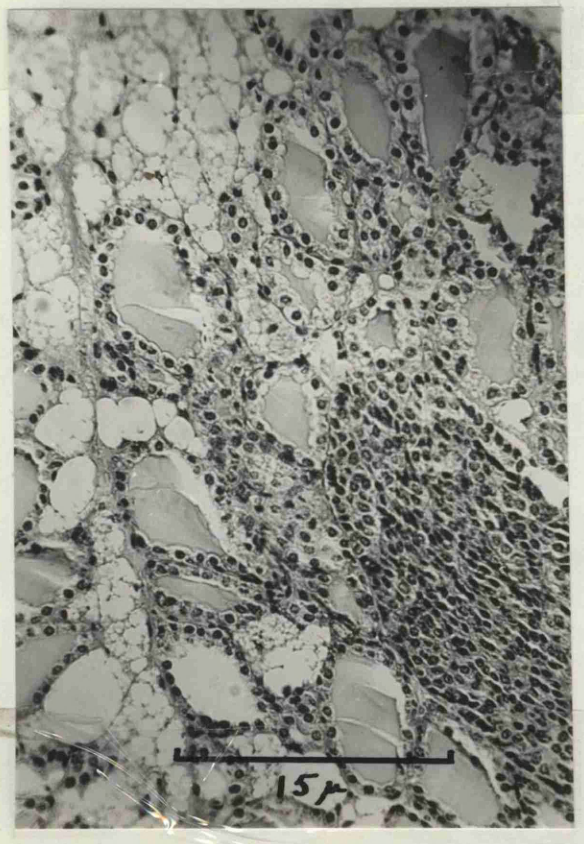

Fig. 51. Thyroid. 3 w. female 21°C control 10 mg. cortisone. H. & E.

Fig. 52. Thyroid. 3 w. female 21°C control 21 mg. ACTH. H. & E.

No obvious change in histological appearance of gland. (p.62)

Appendix C.

Histological methods.

(i) Fixation and embedding

The fixatives used were as follows:

Thyroids, thymus and left adrenal:	Bouin's fluid
Right adrenal:	Formal calcium (10% formaldehyde in 1% $CaCl_2$)
Pituitary:	A modified Bouin containing $\frac{1}{4}$ the normal amount of acetic acid

The Bouin-fixed organs were weighed and then embedded in paraffin wax (M.P. 52°C) and sectioned at 4μ. The right adrenal was embedded in 20% gelatin and frozen sections cut at 8 - 10μ.

(ii) Staining techniques

Paraffin wax sections:	Adrenal and thymus glands were stained with Mayer's haemalum and eosin, or with Mallory's connective tissue stain.
	Thyroid glands were stained with Mayer's haemalum and eosin or with Masson's trichrome stain (with light or fast green).
Gelatin sections:	The right adrenal glands were stained with Sudan black, Sudan 4 and Erlich's haematoxylin, Nile blue sulphate, or with the Schultz stain for cholesterol.

The only staining technique which requires detailed description is the Schultz stain, since this is not well-known.

Schultz stain for cholesterol

Frozen sections (or frozen sections embedded in gelatin) cut at 10μ.

1. Mordant in 2.5% iron alum for 3 days.
2. Rinse in distilled water, mount on slide and blot dry.
3. Add one drop of a mixture of equal parts glacial acetic acid and concentrated sulphuric acid.

In the presence of cholesterol a blue-green colour develops which lasts for about 10 to 30 minutes.

(iii) Numbers of organs examined

	Males		Females	
	Adrenals	Thyroids	Adrenals	Thyroids
Breeding pairs, strain A	4	4	6	6
Adult unmated mice, strain A	10	12	15	18
12-week-old mice, strain A	6	8	7	9
5-week-old mice, strain A	20	21	23	25
5-week-old mice, strain A -3°C controls	6	6	6	6
5-week-old mice, strain GFF	6	5	6	6
3-week-old mice, strain A	18	20	25	28
3-week-old mice, strain A -3°C controls	10	13	12	16
3-week-old mice, injected with ACTH	6	8	5	9
3-week-old mice, injected with cortisone	6	6	9	9

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